RESEARCH ARTICLE

Determination of Nickel as an Elemental Impurity in Atorvastatin Calcium by Atomic Absorption Spectrometer

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ABSTRACT

According to an international conference on harmonization (ICH), elemental impurities can come into drug products from various sources. These elemental impurities thus have to be quantified in drugs. Atorvastatin calcium is a widely used drug to treat cardiovascular diseases. Nickel used as a catalyst during the synthesis process of atorvastatin calcium can cause toxicity to humans and, hence, have to be quantified. A simple, efficient, and accurate, validated method was developed to estimate nickel content as an elemental impurity in atorvastatin calcium using atomic absorption spectroscopy (AAS) with 0.2 nm slit width with a nickel hollow cathode lamp. Acetylene and air mixture was used for flame with 232.0 nm wavelength. The system performance was evaluated by performing the system suitability parameters. The limit of detection and limit of quantification were found to be 0.086 mg/mL and 0.23 mg/mL, respectively. Accuracy studies recoveries were performed at three spiking levels at 50%, 100%, and 150%, and the results were found to be 92.67, 91.33, and 91.00%, respectively. It concludes that according to the United States Pharmacopeia (USP) <232>, this developed and validated atomic absorption spectroscopy method for determining nickel in atorvastatin calcium medication substance was within the permitted limit.

Keywords: Atomic absorption spectroscopy, Atorvastatin calcium, Elemental impurity estimation, Nickel content, USP <232>.

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INTRODUCTION

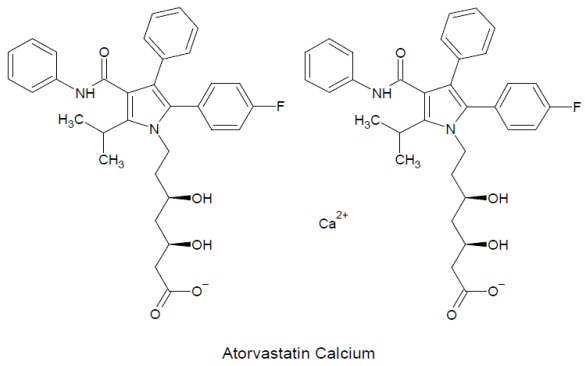
Atorvastatin calcium is an anhydrous calcium salt of atorvastatin.It is made up of atorvastatin as an anion and calcium as a cation in a 2:1 ratio. Atorvastatin calcium is commercially known as Lipitor, is an official drug in united states pharmacopeia (USP).[1] Atorvastatin calcium (Figure 1) is a statin class of drug which prevents cardiovascular disease in patients who have high or abnormal lipid levels in their body.[2] Atorvastatin calcium is used as first-line treatment for cardiovascular diseases. Atorvastatin competitively inhibits HMG-CoA reductase, increasing low-density lipoproteins uptake by hepatocytes, thereby decreasing the low-density lipoprotein-cholesterol in blood. Atorvastatin also increases high-density lipoprotein-cholesterol levels in blood.[2]

Nickel Usage in Synthesis of Atorvastatin Calcium

Nickel as Raney nickel is used as a catalyst in the synthesis process of atorvastatin calcium. According to Kumar *et al.* (2004),[3] nickel is utilized to reduce boronate ester to amino ester form for the intermediate employed in the final synthesis of atorvastatin calcium. A large amount of nickel is required for this reduction process. Boronate ester and nickel are used in a ratio of 1:1, which is a very high quantity of nickel. There is every possibility that nickel as an elemental impurity may be present in large amounts in the end product, which can cause toxicity to the body. Hence, nickel as an elemental impurity must be quantified in atorvastatin calcium (Table 1).[3]

Nickel Toxicity

Nickel, as an elemental impurity, has the potential to be immunotoxic and immunomodulatory, as well as a human allergy.[4]



Atorvastatin Calcium

Figure 1: Structure of Atorvstatin calcium

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Nickel is a carcinogen that can cause multiple types of cancer in humans. It is also haematotoxic, immunotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic, hepatotoxic, according to Kusal *et al* (2018).[4] In its quality guideline, International Council for Harmonisation Guideline, ICH Q3D (R1)[5] categorizes nickel under category Class-2A. This means that nickel has a high chance of occurring in drugs that have been intentionally added during the synthesis process. Hence, the risk assessment has to be done compulsorily.

OBJECTS AND METHODS

According to a survey of the literature, there are a variety of analytical procedures for determining Nickel. Chemical complexing (Andres *et al*., 1995[6]; Garcia Rodriguez *et al*., 1995)[7] or chelating (Hol *et al*., 2011)[8] are two examples of analytical methods. Others include spectrophotometric determination (Bermejo-Barrera *et al*., 1995),[9] differential stripping voltammeter (Yao *et al*., 1990;[10] Zhang *et al*., 1996),[11] and X-ray photoelectron spectroscopy (Biesinger *et al*., 2009)[12] (Salman *et al*., 2011).[13]

Other analytical methods include photochemical vapour generation-batch type ultrasonication assisted gas-liquid separator-atomic absorption spectrometry (Büyükpnar *et al*., 2017),[14] rapid ultrasound-assisted micro-extraction technique (Bahrani *et al*., 2020),[15] laser-induced breakdown spectroscopy (Rifai *et al*., 2020),[16] adsorptive stripping voltammetry (Padilla *et al*., 2021),[17] inductively coupled plasma mass spectroscopy (Bocca *et al*., 2007)[18] and atomic absorption spectroscopy (Srividhya *et al*., 2011)[19] also used for nickel determination.

Nickel analysis using above mentioned was done in a wide variety of samples like water, soil extracts, metal ores, toothpaste, and creams. The literature review also revealed no analytical method to detect and quantify nickel as an elemental impurity in atorvastatin calcium drug where nickel is used as a catalyst for its synthesis. The present work aimed to develop a novel atomic absorption spectroscopy to determine nickel in atorvastatin calcium drug substance and validate the method.

MATERIALS AND METHODS

Reagents

Atorvastatin calcium was obtained as gift sample from SS Pharma, Guntur. Nickel ICP standard 1000 mg/L was purchased from Merck. Nitric acid (AR Grade) and perchloric acid (AR Grade) were purchased from Merck and water used was Milli-Q.

Instrument and Equipment

For this study atomic absorption spectrometer model used was Shimadzu (AA-6300), the analytical balance was of Metrohm make. The hot plate used was of Royal Scientific RSW 127.

Standard Preparation

Nickel (1.0 mL) ICP standard (1000 mg/L) (National Institute of Standards and Technology, NIST traceable material source) solution was diluted in 100 mL with diluents to give a final concentration of 10 mg/L.

Diluent Preparation

Nitric acid (20%) was prepared in sufficient quantity

Preparation of Sample Solution

In a 100 mL beaker, 0.5 g of atorvastatin calcium pharmacological material sample was weighed. 5.0 mL concentrated nitric acid was added to this. After that, 5.0 mL of perchloric acid was carefully added. This was heated on a hot plate until white vapours appeared and the amount was decreased to around 3 mL. After cooling, the mixture was transferred to a 10 mL clean and dry volumetric flask and diluted to the diluent mark.

Preparation of Blank Solution

In a 100 mL beaker, 5 mL of concentrated nitric acid was added to 5.0 mL of concentrated perchloric acid. This mixture was heated on hot plate at 100°C for 10 minutes until white fumes are evolved and till the volume was reduced to about 3.0 mL. The solution was cooled, transferred to a 10 mL volumetric flask, and made up to the volume with diluent.

Analytical Validation Parameters

System Suitability

Five standard nickel solutions were prepared and aspirated into the atomic absorption spectroscopy flame with concentrations of 0.50, 0.75, 1.0, 1.25, and 1.50 mg/L. To assess system appropriateness, averages were generated for triplicate absorbance readings at each standard nickel concentration level, and the correlation coefficient was verified.

Specificity

The specificity of the following samples was tested using atomic absorption spectroscopy: blank, 1.0 mg/L nickel standard, drug sample, and drug ample boosted at 100% level. It was verified

Instrumental Analytical Parameters

Table 1: Optimal operating conditions for flame atomization of Nickel

Analytical parameters	*Setting*
Element	Nickel
Lamp	Nickel Hallow Cathode Lamp
Flame	Acetylene, air
Wavelength	232.0 nm
Slit width	0.2 nm
Calibration type	Linear
Lamp mode	BCG-D2
Lamp current	7 mA
Burner height	7 mm
Burner angle	0°
Fuel gas	1.8 L/min
Support gas	16.0 L/min
Integration time	5.0 sec
Read delay	10 sec
Measurement mode	Absorbance
Sample introduction	Manual aspiration

that there was no interference with other elements and that the spike sample recovery met the accuracy level.

Linearity and Range

The linearity of the proposed method was evaluated by analyzing a series of standard nickel concentrations ranging from 0.50, 0.75, 1.0, 1.25, and 1.50 mg/L, followed by constructing a calibration curve to obtain r^2 value through linear regression analysis.

Limit of Detection (LoD) and Limit of Quantitation (LoQ)

The LoD and LoQ which signifies the sensitivity of the analytical method were calculated by the standard formulae

LoD = 3.3 × Steyx/ Slope of the calibration curve

LoQ = 10 × Steyx/ Slope of the calibration curve

Accuracy

The spiked sample was split into three parts: 50, 100, and 15%. The nickel content of each trial was determined, and the nickel content %recovery in each pathway was calculated.

Precision

The 100% standard was analysed six times in the procedure precision. This demonstrated that the system was consistent, and the percent RSD for six replicates was calculated. The estimated percent RSD values for the aforementioned data should be less than 5% RSD to achieve the acceptance criteria.

Nickel Estimation

The nickel-metal standard and atorvastatin samples were aspirated individually under optimized method conditions. The absorbance of each sample was measured at 232.0 nm wavelength.

$$\text{Total nickel (ppm)} = \frac{A_{\text{Sample}} \times C_{\text{Standard}}}{A_{\text{Standard}}} \times b/a$$

RESULTS

System Suitability

The developed method passes system suitability parameter as the correlation coefficient meets the acceptance criteria (NLT 0.99). The data was presented in Table 2.

Specificity

The following samples were used to test specificity in atomic absorption spectroscopy: a blank, a 1.0 mg/L nickel standard, a drug sample, and a drug sample spiked to 100%. Checked for any interference with any other elements and calculated the % recovery for the spiked sample (Table 3).

Table 2: System suitability values

S. No	*Nickel concentration (mg/L)*	*Absorbance*
1	0.50	0.035
2	0.75	0.057
3	1.00	0.074
4	1.25	0.092
5	1.50	0.114
Correlation coefficient		0.9962

Linearity

A calibration curve was created, and the above plot's linear regression analysis provided a r^2 value of 0.999. This demonstrated the linearity of the developed technique, as seen in Table 4 and Figure 2.

Accuracy

The accuracy of the method was established by spiking known amounts of standard nickel concentrations, i.e., 10, 20, and 30 mg/L, into three individual atorvastatin standard sample solutions. These samples represent three increment levels of 50, 100, and 150%, and each class was aspirated in triplicate. The accuracy recovery values for the three levels mentioned above were 92.67, 91.33, and 91.0%, respectively. This demonstrates the method's accuracy. Data on accuracy can be found in Table 5.

Precision

The absorbance values shown in Table 6 were obtained by repeating the analysis for six times at a concentration of 1.00 mg/L with a nickel standard. The percent RSD value was 2.03, showing that the approach was accurate.

LOD

The researched method was able to detect standard nickel concentrations as low as 0.086 mg/L. The LoD value was

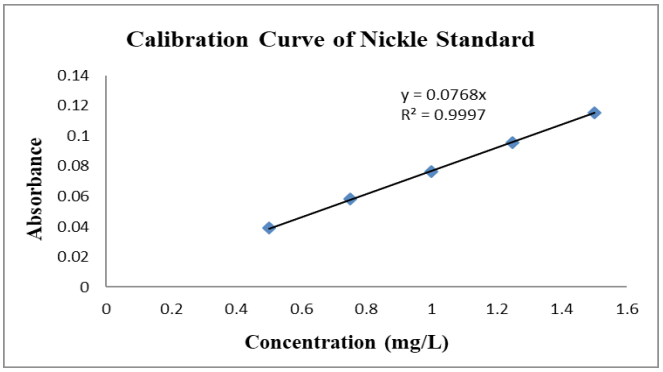

Figure 2: Calibration curve of nickel standard

Table 3: Specificity data

Sample	*Wavelength*	*Absorbance*
Blank solution	232.0 nm	0.005
Nickel standard solution	232.0 nm	0.070
Atorvastatin sample solution	232.0 nm	0.005
100% Spiked sample	232.0 nm	0.067

Table 4: Linearity values

Nickel Standard Concentration(mg/L)	*Absorbance*
0.50	0.0389
0.75	0.0583
1.00	0.0766
1.25	0.0955
1.50	0.1154
Correlation coefficient	0.999

Table 5: Results of Atorvastatin accuracy

S. No	*Name*	*Weight of the sample taken (g)*	*Obtained sample concentration (mg/L)*	*Obtained nickel content (mg/L)*	*Spiked nickel content (mg/L)*	*Obtained recovery (%)*	*Obtained average recovery (%)*
1	50% Spiked sample	0.5009	0.4266	8.5	10	85.00	92.67
		0.5009	0.4729	9.4		94.00	
		0.5009	0.4961	9.9		99.00	
2	100% Spiked sample	0.5006	0.9335	18.7	20	93.50	91.33
		0.5003	0.9649	19.3		96.50	
		0.5009	0.8436	16.8		84.00	
3	150% Spiked sample	0.5006	1.3819	27.6	30	92.00	91.00
		0.5010	1.3533	27.0		90.00	
		0.5011	1.3696	27.3		91.00	

Table 6: Precision results

S.No	*Nickel concentration (mg/L)*	*Absorbance*
1	1.00 mg/ l Nickel standard	0.0845
2	1.00 mg/ l Nickel standard	0.0863
3	1.00 mg/ l Nickel standard	0.0866
4	1.00 mg/ l Nickel standard	0.0875
5	1.00 mg/ l Nickel standard	0.0893
6	1.00 mg/ l Nickel standard	0.0899
Average		0.0874
SD		0.0020
% RSD		2.30

Table 7: Consolidated summary of atorvastatin validation parameters

Parameter	*Result*	*Acceptance criteria*
System suitability	0.9962 (Correlation Coefficient)	NLT 0.99
Linearity range	0.50–1.50 mg/L	-
System Precision	2.30(% RSD)	NMT 5%
% Recovery	91.66	80–120%
LOD	0.086 mg/l	-
LOQ	0.026 mg/l	-
Nickel content in sample	Below Detection Limit	NMT 20 mg/L

0.086 mg/L claiming the sensitivity of the proposed atomic absorption spectroscopy method.

LoQ

The LoQ value was 0.26 mg/L, calculated from the earlier stated formula.

$$LoD = 3.3*0.0020/0.0768 = 0.0859 \text{ (or) } 0.086$$

$$LoQ = 10*0.0020/0.0768 = 0.26$$

DISCUSSION

The developed atomic absorption spectroscopy method was used to determine the amount of nickel in a commercial atorvastatin sample. The nickel concentration in the bulk sample was found to be below the detection limit. Before beginning any validation parameter, the system suitability parameter was run to guarantee that the atomic absorption spectrometer produced accurate findings. The correlation coefficient is a measure that indicates whether the instrument is responding optimally. Nickel was measured both in a blank matrix and in a sample matrix. As a result of the lack of influence from both matrices, the approach was determined to be specific. The method precision validation parameter specifies the degree to which measurements agree with one another, as measured in percentage relative standard deviation. The outcome was deemed to meet the required standards.

The system suitability parameter was executed before starting any validation parameter to ensure the atomic absorption spectrometer gives the correct results. The correlation coefficient is the parameter that indicates that the instrument is giving the optimum response. Nickel was quantified in a blank matrix as well in the presence of a sample matrix. There was no interference from both the matrices, and hence the method was found to be specific. Method precision validation parameter indicates the closeness of the measurement in agreement with each other, measured in percentage relative standard deviation. The result was found to be within acceptance criteria. Linearity studies are performed to define the range of the method. The atorvastatin calcium AAS method was found to be linear for the above-mentioned range.

Limit of detection (LoD) indicates the least concentration of analyte that the method can detect. Limit of quantitation (LoQ) gives the smallest amount of analyte the method can measure accurately. Limit of detection and limit of quantitation parameters shows the sensitivity of the method. LoD and LoQ of the method was determined from the calibration curve. The result was found to be within acceptance criteria. The overall summary of all the validation parameters and their acceptance limits were summarized in Table 7.

CONCLUSION

Atorvastatin calcium is a commonly prescribed medication for the treatment of cardiovascular disorders. Because nickel, which is employed as a catalyst in the manufacture of Atorvastatin calcium, can be harmful to humans, it must be measured. This study used a validated easy, precise, and exact atomic absorption spectroscopy approach to assess nickel as an

elemental contaminant in atorvastatin calcium pharmaceutical drugs. The maximum permitted nickel concentration was 20 ppm, according to USP General Chapter <232>. This simple, cost-effective, and exact method can be used to determine the nickel content of atorvastatin calcium.

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