Research Article

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Direct determination of amoxicillin sodium in its pure and dosage forms using atomic absorption spectrometry based on its sodium content

Abstract

Atomic absorption spectrometric method has been developed and validated for direct determination of amoxicillin sodium in its pure and pharmaceutical dosage forms based on its sodium content at λ589 nm. The developed method was found to be time and cost saving and has no tedious or complex procedures. The developed method is very sensitive regarding LOD (0.025ppm of sodium equivalent to 0.423µg mL^{-1}), LOQ (0.076ppm of sodium equivalent to 1.282µg mL^{-1}) and linearity range (0.2-1ppm of sodium) equivalent to (3.37-16.84 µg mL^{-1}). The accuracy of the method was further assessed by application of standard addition technique and very satisfactory results were obtained. The results obtained by the proposed method were compared by statistical measures to reference method and there were no significant difference between the developed method and the reference method regarding the accuracy and precision. The developed method was validated according to ICH guidelines and the results were satisfactory.

Keywords: amoxicillin, atomic absorption spectrometry, direct, guidelines, radiation, sensitivity

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Abbreviations: AAS, atomic absorption spectrometry; AMX, amoxicillin sodium; LOD, limit of detection; LOQ, limit of quantitation; RSD, relative standard deviation

Introduction

The technique of atomic absorption spectrometry (AAS) is one of the most powerful methods of analysis, because of the absorption of the highly selective resonance radiation and high sensitivity which allowed for trace element determinations in powders,[1] pure drugs, pharmaceuticals,[2] and many biological fluids.[3]

The main goals of analytical atomic absorption spectrometry are to attain the lowest limits of detection (down to single atoms), use the broadest dynamic range, suppress the matrix effect, eliminate spectral interferences, minimize the time and cost required for sample preparation.[4]

The environmental safety in atomic absorption spectrometry is of great importance, since during the whole procedures the only and one solvent use is just a sterile water taking in consideration the absence of hazardous, refluxing reactions and the use of any toxic reagents.

Amoxicillin sodium [AMX], Figure 1, chemically known as 6-(p-hydroxy-alpha-amino phenyl acetamido) penicillanic acid. Its molecular weight is 387.386 and its molecular formula is $C_{16}H_{18}N_3NaO_5S$. It is a white powder with sulphurous odour and has a water solubility of 958mg mL^{-1}.[5,6]

It is a broad spectrum antibiotic used in the treatment of infections caused by both gram-positive and gram-negative bacteria, specially tonsillitis, dental abscess, osteomyelitis and upper respiratory tract infections.[5,6] The literature review revealed that several analytical methods have been reported for the determination of AMX in its pure and pharmaceutical dosage forms using spectrophotometry,[7-10] HPLC[11] and LC-MS.[12,13]

Despite the presence of many analytical techniques for determination of AMX, the novelty of the present work is that; none of the published methods bring all the advantages of sensitivity, simplicity, time and cost saving and being environmentally safe together.

The main aim of this work is to develop a simple, sensitive and inexpensive AAS method for the determination of amoxicillin-Na in its pure and dosage forms through estimation of its Na content.

Experimental

Pure sample

Pure amoxicillin sodium certified by the manufacturer to contain (99.46 %) was pursued as a gift sample from EIPICO Company, 10th of Ramadan city, industrial zone B1, Cairo, Egypt.

Pharmaceutical preparation

E-MOX® vials (B.No 1707849) labelled to contain 1000 mg amoxicillin-Na per vial, manufactured by EIPICO Company, 10th of Ramadan city, industrial zone B1, Cairo, Egypt. And purchased from local market.

Chemicals and reagents

Water used throughout the procedure was freshly double distilled.

Apparatus

A Thermo Elemental Atomic Absorption Flame Spectrophotometer, (Cambridge-UK) serial no. JE710572 computed with solar data station software version 9.03. Sodium was measured at wavelength 589nm, band pass 0.5nm, relative noise 1.0nm, lamp current 10mA, and integration time 5 second.

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Standard solutions

Stock solution of the drug (0.1mg mL^{-1}) was prepared by dissolving 10mg of amoxicillin-Na in 50mL double distilled water and the volume was completed to 100mL with the same solvent.

Procedures

General procedure

Aliquots of the standard amoxicillin-Na solution (0.1mg mL^{-1}) containing (33.7-168.4µg) of the drug equivalent to (2-10ppm Na) were transferred into a serious of 10mL volumetric flasks, completed to the mark with double distilled water. The drug was determined through its Na content at 589nm.

Validation of the procedure[14,15]

The method was tested for linearity, limits of detection and quantitation, accuracy and precision.

Procedure for pharmaceutical preparation

Five E-MOX® vials each labelled to contain 1000mg amoxicillin-Na were weighed and mixed carefully. An accurately weighed quantity of the powder equivalent to 10mg of amoxicillin-Na was introduced into a 100mL volumetric flask, extracted with 50mL double distilled water by shaking for 5 minutes. The volume was completed to the mark with the same solvent, then filtered to obtain a solution labelled to contain (0.1mg mL^{-1}) to be analyzed by the proposed method. The drug concentrations were calculated from the corresponding regression equation.

Reported method[10]

The method based on direct determination of amoxicillin using citro phosphate buffer pH 7.2 at 231nm.

Results and discussion

In the present study, a simple and sensitive Atomic Absorption Spectrometry (AAS) procedure was suggested for quantitative determination of amoxicillin-Na through its Na content at λ589 nm

    a. Method validation

    b. Linearity and range

Under the described experimental conditions, the calibration graph for the method was constructed by plotting the absorbance values versus drug concentrations in µg mL^{-1}.

The regression plot was found to be linear over the range of (3.37-16.84)µg mL^{-1} of amoxicillin-Na equivalent to (0.2-1)ppm Na; as shown in Figure 2.

Linearity range, regression equation, intercept, slope and determination coefficient for the calibration data were presented in Table 1.

Limits of detection and quantitation

The limit of detection (LOD) and the limit of quantitation (LOQ) were calculated from the following equations:

    LOD = 3.3 σ/S

    LOQ = 10 σ/S

Where σ: is the residual standard deviation of a regression lines.

S: is the slope of the calibration curve.

LOD and LOQ values were calculated for the proposed procedures and the obtained results indicated the sensitivity of the proposed method as shown in Table 1.

Table 1 Regression and validation data for determination of amoxicillin-Na by the proposed atomic absorption spectrometry method

Parameters		Atomic absorption spectrometry
Wavelength (nm)		589
Linearity range	ppm	0.2-1
	(µg mL^{-1})	3.37-16.84
Regression Equation		y^a = bx^b+a
Slope (b)		0.9001
Intercept (a)		0.0249
Determination coefficient (r^2)		0.9994
Accuracy (%R)^c		101.42
Precision (%RSD)	Repeatability ^{c}	1.297
	Intermediate precision d	1.744
LOD	ppm	0.025
	(µg mL^{-1})	0.423
LOQ	ppm	0.076
	(µg mL^{-1})	1.282

^{a}Absorbance of Na at 589nm.
^{b}Amoxicillin concentration in (µg mL^{-1}).
^{c}Values for 3 determinations of 3 different concentrations.
^{d}Values for 3 determinations of 3 different concentrations in 3 Successive days.

Accuracy and precision

Accuracy and precision of the method were determined by applying the proposed procedure for determination of three different concentrations, each in triplicate, in their pure form in the same day (intra-day) and in three successive days (inter-day), then the accuracy as percent recovery (%R) and precision as percent relative standard deviation (%RSD) were calculated.

The values of %R confirms excellent accuracy. Moreover, the small values of %RSD indicate high precision of the method, as shown in Table 1.

Specificity

The standard addition technique was applied to check the specificity of the described method by adding known quantities of the studied drug in its pure form to already analyzed pharmaceutical preparation and the percent recovery of the pure added was calculated. The data listed in Table 2 indicates no matrix interference.

Pharmaceutical applications

The proposed method was applied for the selective determination of amoxicillin-Na in E-MOX® vials. Satisfactory results were obtained

Direct determination of amoxicillin sodium in its pure and dosage forms using atomic absorption spectrometry based on its sodium content

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in good agreement with the label claim. The obtained results were statistically compared to those obtained by the reported method.[10] No significant differences were found by applying t-test and F-test at 95% confidence level,[16] indicating good accuracy and precision of the proposed methods for the analysis of the studied drugs in its pharmaceutical dosage form, as shown in Table 3.

Table 2 Recovery study of amoxicillin-Na by adopting standard addition technique via the proposed Atomic absorption spectrometry method

Pharmaceutical(ppm)		Pure (ppm)		%Recovery
Taken	Found*	Added	Found	
		0.2	0.2033	101.65
0.4	0.4067	0.4	0.4043	101.08
		0.6	0.6051	100.85
Mean				101.19
%RSD				0.417

Table 3 Determination of Amoxicillin-Na in E-MOX® vials by the proposed Atomic absorption spectrometry and reported methods

Parameters	Atomic absorption spectrometry	Reported method (10)
Na	5	5
Mean	101.69	98.74
Variance	0.481	0.233
%RSD	0.694	0.489
Student's t-test (2.306)[b]	1.076	–
F-value (6.39)[b]	2.064	–

[a]Number of measurements
[b]The values in parenthesis are tabulated values of "t" and "F" at (P=0.05).

Conclusion

In this work, Atomic absorption spectrometric technique provides the estimation of AMX in bulk and pharmaceutical dosage form. The method has the advantage of being simple, sensitive, accurate and time, cost saving. The method could be applied for routine analysis of pure AMX or in its pharmaceutical formulation and could also be easily used in quality control laboratory for its analysis. The methods are also suitable and valid for application in laboratories lacking liquid chromatographic instruments.

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Conflict of interest

Author declares there is no conflict of interest.

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