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Analytical Method Validation of Azadirachtin Extracted From Azadirachta Indica

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Abstract:

A simple UV spectrophotometric method for the determination of Azadirachtin is developed exhibiting maximum absorbance using distilled water at 542nm.The present study is based on the detection of sample drug concentration using dichloro methane as diluent. All the conditions required were optimized. Statistical analysis was carried out and results of which were satisfactory. The optical characteristics such as absorption maxima, regression analysis and correlation coefficient are obtained. Recovery studies were close to 100% that indicate the accuracy and precision of proposed method and the non-interference of the formulation excipients. All the valid parameters are summarized.

1. Introduction

Azadirachta indica, otherwise known as Neem, Neem tree and Indian Lilac belong to the family Meliaceae. It is local to India and the Indian subcontinent including Nepal, Pakistan, Bangladesh and Sri Lanka. It is typically grown in tropical and semi-tropical districts. Neem trees now likewise develop in islands situated in the southern part of Iran. Its fruits and seeds are wellspring of Neem oil (Nanduri, S 2003). Azadirachtin is limonoid triterpene found in neem tree (Pandreka et al., 2015). It has insect growth inhibition, antifeedant, growth-regulating and development-modifying properties (Abdelouahed Alouani et al., 2009).

2. Methodology

2.1 UV-visible-spectrophotometric method for the estimation of Azadirachtin

A simple UV spectrophotometric method for the determination of Azadirachtin is developed exhibiting maximum absorbance using distilled water at 542nm, phosphate buffer pH 2.5 at 313nm and HCl:H_2O in 1:9 ratio at 540nm.

Spectrophotometric parameter is established for the standardization of methods including statistical analysis of the drug. These methods have been successfully extended to pharmaceutical dosage forms.

2.2 Instrument

Soxhlet set up, Labindia Double beam UV / Visible spectrophotometer

2.3 Extraction method

Ripe neem fruits were obtained from local vegetable market in Hyderabad. Higher concentration of bitterness is found in neem kernel. It is thus essential to understand fruit collection and depulping to get kernels.

Neem yields fruits during May to August, thus fruits were collected and depulped as early as possible preventing the contact of fruits with soil.

Depulping is a process to remove seed coat and pulp from the neem seed. It is done by hand and using mechanical depulper developed by Neem Research and Technology Development Centre (NRTDC). If processed properly these neem seeds can be stored for about 6-12 months.

Separation of the kernels**:**

The dried neem seeds are ground slightly with hand and outer shell is removed, kernels are present inside the shell which are separated and made into powder using a

grinder. It should be pounded such that no oil comes out of it. This coarse powder is used for further studies of the extraction of Azadirachtin.

Extraction of Azadirachtin form neem kernel powder:
For extraction of Azadirachtin by solvent process, weigh about 500g of clean neem kernel powder. It should be pounded in such a way that no oil comes out of it. Make a thimble and fill it with kernel powder and place it in a Soxhlet apparatus and add about 600ml of di-chloro methane. Keep it on a heating mantle and heat to reflux for about 12 hours. When the powdered kernel is extracted with solvents like di-chloro methane or ethyl acetate, liminoids and other constituents get dissolved in it, leaving the seed cake without any active components. The solvent from the mixture is recovered by distillation. The distilled or concentrated solution is kept aside for cooling. Hexane is added to the concentrate and then filtered using vacuum pump. When the above residue is dried it gives a pale greenish colored powder. This powder consists of Azadirachtin and a very small quantity of Nimbin. 100g of neem kernel powder on extaction gives about 1 gm of Azadirachtin (Supriya Dubhashi et al., 2013).

2.4 Reagents and standard –Azadirachtin tablet

a. Azadirachtin working standards
b. Dichloromethane

2.5 Diluent preparation

Dichloro methane was used as diluents.

2.6 Procedure

2.6.1 Preparation of stock solution

A standard stock solution containing 1mg/ml that is 1000µg/ml is prepared by dissolving 100mg of Azadirachtin in 100ml distilled water.

2.6.2 Preparation of working standard solution

A working standard containing 100µg/ml is prepared by diluting the above stock solution taking 1ml in 100ml distilled water.

2.7 Optimization of λ max

The above prepared solution was scanned in the UV/Visible region (800-200nm) for the absorbance values to get λmax.
The λmax was found to be 542 nm.

2.8 Assay-content estimation

2.8.1 Calibration curve

Aliquots of standard solution from 5 to 25mcg/ml were prepared and diluted as required. The wavelength is measured at 542nm. The calibration curve that is linear is computed by taking above data.

2.8.2 Sample solution

Isolated sample containing Azadirachtin compound were successfully analyzed by the proposed method. Isolated sample of Azadirachtin was accurately weighed; powder equivalent to 154.75mg of Azadirachtin was dissolved in a 250ml volumetric flask and filtered. The solution was suitably diluted and analyzed as given under the procedure for bulk samples. The results were represented in table 1.

3. Method Validation

3.1Precision

3.1.1 Preparation of standard solution:

Accurately weigh and transfer 10mg of Azadirachtin working standard into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Measure the absorbance of the Azadirachtin standards for five times and calculate the %RSD. The %RSD for the five replicate absorbance was found to be within the specified limits.

Table 1: Assay of azadirachtin in obtained sample

Labeled Amount (mg)	Obtained Amount By Proposed Method (mg)	% Recovery By Proposed Method	Mean Recovery %
5.0 mg	5.08	101.7%	100.05%
10.0 mg	9.92	99.26%	
15.0 mg	14.87	99.14%	

Table 2: Optical characteristics and validation data of Azadirachtin

Parameters	Results
Lambda Max [λ max]	542 nm
Regression Equation [Y] **Slope [m]** **Intercept [c]**	Y = 0.017x+0.001 0.017 0.001
Correlation Coefficient [R^2]	0.999
%RSD	1.777
LOD	0.591
LOQ	1.970

3.1.2 Intermediate precision/ruggedness

To evaluate the intermediate precision (also known as Ruggedness) of the method, precision was performed on different day by using same dimensions.

Preparation of Standard Solution:

Accurately weigh and transfer 10 mg of Azadirachtin working standard into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Further pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

3.2 Accuracy

3.2.1 Preparation of standard stock solution

Accurately weigh and transfer 10 mg of Azadirachtin working standard into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

3.2.2 Preparation sample solutions

For preparation of 50% solution (With respect to target assay concentration)

Accurately weigh and transfer 5.0mg of Azadirachtin sample into a 100ml volumetric flask add about 70 ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target assay concentration)

Accurately weigh and transfer 10.0mg of Azadirachtin sample into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration)

Accurately weigh and transfer 15.0mg of Azadirachtin sample into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution). Further pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

Measure the absorbance of the standard solution, accuracy -50%, accuracy -100% and accuracy -150% solutions. Calculate the amount found and amount added for Azadirachtin, calculate the individual recovery and mean recovery values.

3.3 Linearity

3.3.1 Preparation of stock solution

Accurately weigh and transfer 10 mg of Azadirachtin sample into a 100ml volumetric flask add about 70ml of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent (Stock solution).

Level – I: Preparation of 5µg/ml Azadirachtin solution

Pipette 0.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – II: Preparation of 10µg/ml Azadirachtin solution

Pipette 1.0ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – III: Preparation of 15µg/ml Azadirachtin solution

Pipette 1.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – IV: Preparation of 20µg/ml Azadirachtin solution

Pipette 2.0ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

Level – V: Preparation of 25µg/ml Azadirachtin solution

Pipette 2.5ml of the Azadirachtin stock solution into a 10ml volumetric flask and dilute up to the mark with diluents.

3.4 Robustness

As part of the Robustness, deliberate change in the wave length was made

The wavelength was varied at 540 nm to 542 nm.

Standard solution 6ppm of Azadirachtin was prepared and analysed using the varied wave length along with method wave length.

On evaluation of the above results, it can be concluded that the variation in wave length affected the method significantly. Hence it indicates that the method is robust even by change in the wave length ±1

The method is robust only in wave length condition.

Table 3: Optical characteristics and validation data of azadirachtin upon first-order derivatization

Parametres	Results
Lambda Max [λ max]	542nm
Regression Equation [Y] **Slope [m]** **Intercept [c]**	Y = 0.010x+0.002 0.010 0.002
Correlation Coefficient [R^2]	0.999
%RSD	0.339
LOD	0.165
LOQ	0.55

Table 4: Assay of isolated azadirachtin

Labeled Amount (mg)	Obtained Amount By Proposed Method (mg)	% Recovery By Proposed Method	Mean Recovery %
5.0 mg	4.97	99.4%	99.8%
10.0 mg	10.08	100.8%	
15.0 mg	14.9	99.6%	

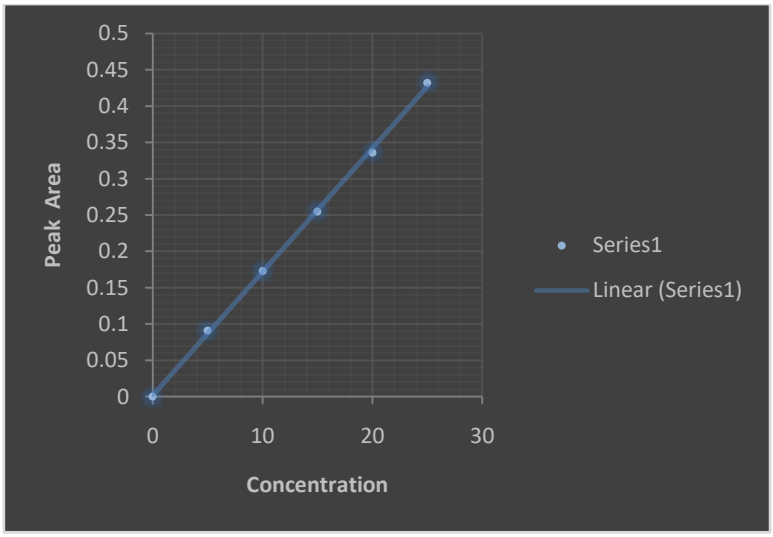

Figure 1: Linearity of isolated sample of Azadirachtin in dichloromethane

Table 5: Calibration curve for isolated sample of Azadirachtin

Concentration(µg)	Peak Area
5	0.091
10	0.173
15	0.225
20	0.336
25	0.432

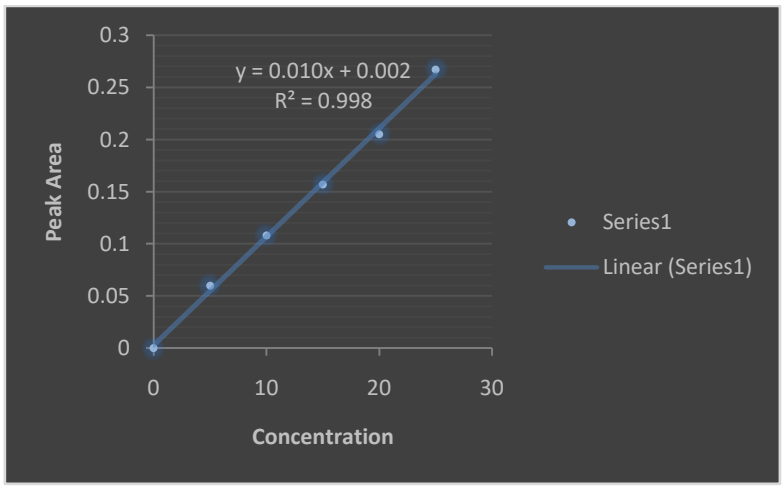

Figure 2: Linearity of Azadirachtin pharmaceutical dosage form in dichloro methane

Table 6: Calibration curve for azadirachtin pharmaceutical dosage form

Concentration(µg)	Peak Area
5	0.06
10	0.108
15	0.157
20	0.205
25	0.267

Table 7: Content determined using individual isolated sample of Azadirachtin as calibrants

Azadirachtin Sample conc.	Calibration With commercial product	Calibration with individual azadirachtin
5	0.06	0.091
10	0.108	0.173
15	0.157	0.225
20	0.205	0.336
25	0.267	0.432

Table 8: Claimed and actual purity of commercial Azadirachtin (Lot 1) and individual Azadirachtin (Lot 2) samples

Azadirachtin samples	Supplier Purity Claim (%)	Determined Purity (%)	
		Lot 1	Lot 2
5	>97%	95.6	95.8
10	>97%	97.7	96.7
15	>97%	87.1	66.1
20	>97%	83.4	94.2
25	>97%	78.3	69.1

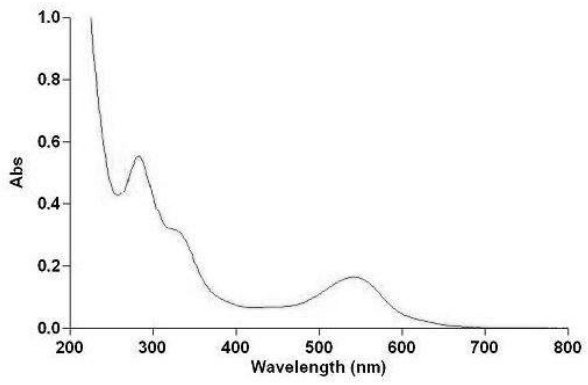

Figure 3: Assay of isolated sample of Azadirachtin

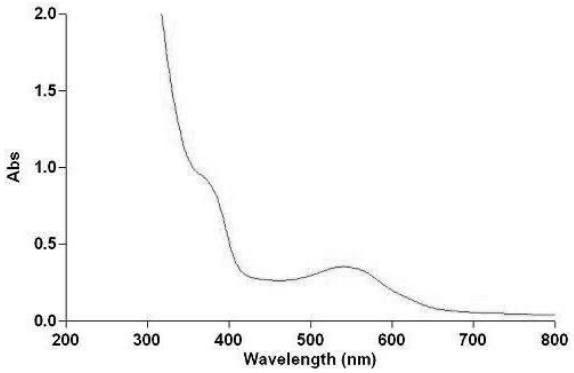

Figure 4: Assay of Azadirachtin pharmaceutical dosage form

Table 9: Robustness

S.No	Wave length(nm)	Absorbance
1	540nm	0.191
2	542nm	0.188
3	550nm	0.185

4. Conclusion

In the present study, Azadirachtin a phytochemical constituent extracted from kernels of Azadirachta Indica was collected and a simple, precise method was developed for the raw material.

The method for raw material of Azadirachtin was found to be precise when compared to a finished product Azadirachtin pharmaceutical dosage form.

Conflict of interest

None declared

5. References

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