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Quality Control And Standardization Of Azadirachtin In Herbal Medicines By Spectroscopic And Chromatographic Techniques

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Abstract:

Background: Many herbal medicines and their formulations containing Azadirachtin are available in market and hence quality control of Azadirachtin in is very important and essential in manufacturing industries

Main body of the abstract: We have reviewed various scientific research published on quality control analysis and standardization of Azadirachtin in its isolated form, extract or any other herbal or polyherbal preparation. We have mainly focused on the spectroscopic and chromatographic methods for qualitative and quantitative analysis of Azadirachtinand they were comprehensively presented in the present review work.

Short conclusion: The present review concludes that the spectroscopic and chromatographic methods play great role in the quality control and standardization of Azadirachtin in its isolated form, extract and in its herbal and polyherbal preparation.

Key words: Azadirachtin, Analytical techniques, Estimation, Neem

Introduction:

The study of pharmacognosy, phytochemistry, and the application of medicinal plantswhich serve as the basis for conventional medicine or drugs, comes under the umbrella of herbal medicine. Ayurveda, Siddha, and Unani systems of medicine make up the majority of Indian medical systems [1].

The herbal drug or active ingredient used to treat numerous disorders is called azadirachtin. The primary ingredient that is derived from neem seeds, leaves, and bark is azadirachtin (Figure 1). A lovely broad-leaved evergreen tree, the neem tree is considered to have originated in Burma. The active ingredient of neem, Azadirachta indica, which is a member of the Meliaceae family, is azadirachtin. Azadirachtin is a tetranortriterpenoid that is extensively oxidized and a member of the limonoid family[2].

The primary component of neem, azadirachtin, is utilized as a broad range pesticide, anti-feedant, anti-cancer, anti-fungal, as well as to purify and detoxify the blood. There are two types of azadirachtin: azadirachtin A and azadirachtin B. Azadirachtin is the active component in a variety of formulations, including the Geo-Fresh Organic Neem pill, the Himalaya Neem Skin Wellness tablet, the Vestige Neem capsules, the Organic Aura Neem Leaf capsules, the BAPS AmrutNeem capsules, and others[3-4].

Very few analytical techniques have been described by the researchers for estimating azadirachtin in commercial formulations. Modern analytical tools are extremely important for the standardization and quality control of herbal medicines. In particular, spectroscopic and chromatographic techniques are crucial for the quality assurance and scientific verification of herbal remedies[5]. The stated methods own drawbacks include the usage of pricy and risky solvents and the fact that the results haven't been fully confirmed. Azadirachtin has been a crucial active component in numerous formulations that have strong pharmacological activity.

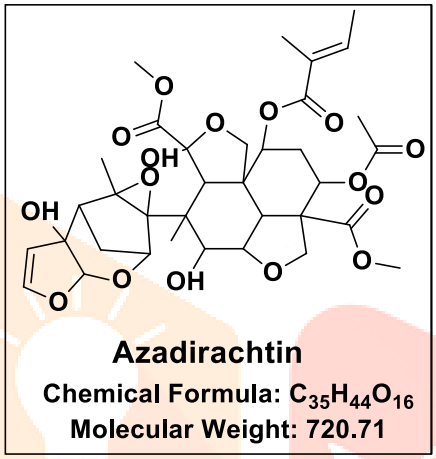

Figure 1: Chemistry of Azadirachtin

Methods:

Many herbal medicines and their formulations containing Azadirachtin are available in market. We have reviewed various scientific research published on quality control analysis and standardization of Azadirachtin in its isolated form, extract or any other herbal or polyherbal preparation. In the present review article, we have mainly focused on the different spectroscopic and chromatographic methods. The detailed summery of spectroscopic standardization and chromatographic techniques of azadirachtin presented in Table 1.

Author name	Title of work	Description of analysis
S Srivatsava et al.	In vitro azadirachtin production. In Bioactive molecules and medicinal plants	Reverse phase-HPLC for the qualitative and quantitative estimation of Azadirachtin at 217nm.
Atmakuru Ramesh et al.	Rapid preconcentration method for the determination of azadirachtin-A and-B, nimbin and salannin in neem oil samples by using graphitized carbon solid phase extraction.	The azadirachtin-A and -B, nimbin and salannin were eluted with 5 ml of acetonitrile and was quantified using HPLC with UV detection.
Moacir Rossi Forim et al.	Simultaneous quantification of azadirachtin and 3-tigloylazadirachtol in Brazilian seeds and oil of Azadirachta indica: application to quality control and marketing.	Quantification of Azadirachtin and 3-tigloylazadirachtol in Brazilian seeds of Azadirachta Indica using a rapid, sensitive and selective HPLC-UV and HPLC-MS/MS method by mobile phase MeOH: acetonitrile: THF: H_2O (36.75: 7.35: 4.9: 51) at 217nm.
Sara R. Fernandes et al.	Chemistry, bioactivities, extraction and analysis of azadirachtin: State-of-the-art.	Analysis of Azadirachtin by using separating HPLC technique. After extracting the Azadirachtin from the seeds, fruits and etc. at 214-220nm.
Nisha Dahiya et al.	In vitro and ex vivo activity of an Azadirachta indica A. Juss. seed kernel extract on early sporogonic development of Plasmodium in comparison with azadirachtin A, its most abundant constituent.	characterization and isolation of the neem to get Azadirachtin. By carrying out the NMR and Mass analysis they have found one of their analyzed fractions as mixture of Azadirachtin A and B. After that purification is done by HPLC using the mobile phase n-hexane: Ethyl acetate (1:1)
OtmarSchaafet al.	Rapid and sensitive analysis of azadirachtin and related triterpenoids from neem (Azadirachta indica) by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry.	Analysis is done by HPLC using the mobile phase acetonitrile:0.1% trifluoroacetic acid (80:20) at 217nm.
O.J. Pozo et al.	Determination of abamectin and azadirachtin residues in orange samples by liquid chromatography–electrospray	Development of a rapid and sensitive LC–ESI-MS–MS method for the determination of azadirachtin and abamectin residues in orange samples

	tandem mass spectrometry.	
Deepak Sharma et al.	Development and Validation of Stability Indicating UV-Visible Spectrophotometric Method for Simultaneous Estimation of Neem (Azadirachtin) and Curcumin in Pharmaceutical Tablet Dosage Form.	To simultaneously estimate azadirachtin and curcumin complex in combined pharmaceutical tablet dose form, a stability indicating UV Visible spectrophotometric method must be developed and validated by combination at two wavelengths, 271 nm and 421 nm.
Steven V Ley et al.	"Conxmtivist" Approach to Organic Structure Determination Lsd-Programme Assisted NMR Analysis of the infect Antifeedant Azadirachtin.	Rapid column chromatography on silica gel was used to isolate pure azadirachtin from a crude sample (309) of Neem seed extract employing a 7:3 ratio of ethyl acetate: petroleum ether as the solvent system.
Hui-p1ng huang et al.	Analysis of azadirachtin by supercritical-fluid chromatography.	supercritical-fluid chromatography to quantitatively determine the natural insecticide azadirachtin in crude neem seed extract, with detection by UV absorption at 210–220 nm and utilizing carbon dioxide: methanol as the mobile phase. 10 mg at 212 nm.
R. Thejavathi et al.	Determination of azadirachtin by reversed-phase highperformance liquid chromatography using anisole as internal standard.	Determination of azadirachtin by reversed-phase highperformance liquid chromatography using anisole as internal standard. Using anisole as the internal standardby reversed-phase HPLC method.
María Nella Gai et al.	An HPLC Method for Determination of Azadirachtin Residues in Bovine Muscle.	High-performance liquid chromatography (HPLC) to measure the presence of azadirachtin (A and B) residues in beef muscle by water: acetonitrile mixture (27.5:72.5, v/v) at 215nm.
J. D. Warthen Jr et al.	Estimation of Azadirachtin Content in Neem Extracts and Formulations.	High-performance liquid chromatographic reversed-phase procedure has been developed whereby azadirachtin content can be estimated in crude extracts of neem and in dust formulations of neem at 217nm.
K.M.S. Sundaram et al.	High performance liquid chromatographic method for the analysis of azadirachtin in two commercial formulations and neem oil.	Azadirachtin (AZ) content in neem formulations and neem oil can be determined using an enhanced high performance liquid chromatographic technique by Hexane:dichloromethane (60:40)at 210nm.

K.M.S. Sundaram et al.	High Performance Liquid Chromatographic Determination of Azadirachtin in Conifer and Deciduous Foliage, Forest Soils, Leaf Litter and Stream Water.	The measurement of azadirachtin (AZ-A) in conifer and deciduous foliage, forest soil, litter, and stream water are detailed using a high-performance liquid chromatographic (HPLC) approach at 210nm.
Reza Farjaminezhad et al.	Establishment of green analytical method for ultrasound-assisted extraction of azadirachtin, mevalonic acid and squalene from cell suspension culture of Azadirachta indica using response surface methodology.	In this study, azadirachtin, mevalonic acid, and squalene extraction from A. indica cell suspension culture was optimized using response surface approach. The Box-Behnken Design was applied to the factor optimization. HPLC-DAD identified the azadirachtin, mevalonic acid, and squalene.
Joelma Abadia Marciano de Paula et al.	Ultrasound-assisted extraction of azadirachtin from dried entire fruits of Azadirachta indica A. Juss. (Meliaceae) and its determination by a validated HPLC-PDA method.	Ultrasound-assisted extraction of azadirachtin from dried entire fruits of Azadirachta indica A. Juss. (Meliaceae) and its determination by a validated HPLC-PDA method. Acetonitrile: water (40:60) at 214nm.
Mohibb e azam et al.	Estimation of Azadirachtin-A Content of Emulsifiable and Solution Concentrates of Neem.	A column chromatographic cleanup technique was created to get rid of the various surfactants and aliphatic and aromatic solvent components included in prepared neem. The silica gel (60-120 British Standard Sieve) and 250 mL of hexane-acetone (6 :4) were placed in a glass column.
Penmatsatanuja et al.	Development and Evaluation of Thin-Layer Chromatography-Digital Image-Based Analysis for the Quantitation of the Botanical Pesticide Azadirachtin in Agricultural Matrixes and Commercial Formulations: Comparison with ELISA.	Analyzing azadirachtin in processed commercial pesticide formulations and spiked food matrixes while utilizing acidified vanillin reagent as a post chromatographic derivatizing agent helped to confirm the procedure.
Pierluigicaboni et al.	Persistence of Azadirachtin Residues on Olives after Field Treatment.	The acetonitrile/water gradient elution method that was used enabled azadirachtin to be separated chromatographically.

Jianming Dai et al.	Extraction and Colorimetric Determination of Azadirachtin-Related Limonoids in Neem Seed Kernel.	The best circumstances for carrying out the assay were chosen through the examination of numerous elements impacting the sensitivity of detection, including the concentration of vanillin, acid, and the time needed for color production by HPLC method at 577nm.
P. T. Deota et al.	Estimation and isolation of azadirachtin-a from neem [Azadirachta indica a. Juss] seed kernels using high performance liquid chromatography.	On a reverse phase C18, analytical HPLC-UV was performed using a solvent solution of methanol and water (60:40 v/v) at 217nm.
J.K. Lalla et al.	Azadirachtin as a Biomarker Compound in HPTLC Assay of Seed and Seed Oil of Azadirachta indica A. Juss.	Azadirachtin as a Biomarker Compound in HPTLC Assay of Seed and Seed Oil of Azadirachta indica A. Juss at 677nm.
Priyanka Srivastava et al.	Increased production of azadirachtin from an improved method of androgenic cultures of a medicinal tree Azadirachta indica A. Juss.	The azadirachtin, a tetranortriterpenoid, demonstrated HPLC elution at 6.39 min in the standard and sample extracts that were made using the materials and techniques methodology.
Nutan Kaushik et al. have	Determination of azadirachtin and fatty acid methyl esters of Azadirachta indica seeds by HPLC and GLC	Technique has been created to gauge the fatty acid composition and azadirachtin content of neem kernels. Crushed neem kernels are steeped in ethanol for an entire night being analysed by HPLC. Acetonitrile-water (40:60) at 214nm.
De menezes et al.	Determination of biopesticide azadirachtin in samples of fish and in samples of water of fish ponds, using chromatography liquid of high performance.	Determination of biopesticide azadirachtin in samples of fish and in samples of water of fish ponds by HPLC method.

Table 1: Spectroscopic standardization of Azidirachtin in herbal medicines

Spectroscopic and chromatographic techniques:

S Srivatsava *et al*.,have reported the extraction procedures of Azadirachtin.They have used different organic solvents for extraction like methanol, ethanol, n-hexane, ethyl acetate and dichloromethane. They have usedReverse phase-HPLC for the qualitative and quantitative estimation of Azadirachtin. As Azadirachtin does not carry a strong ultraviolet (UV)-absorbing chromophore. The UV signalswere recorded at lower wavelength at 217 nm. Authors also reported thesuper-critical fluid chromatography and liquid chromatography-mass spectrometry forthe analysis of Azadirachtin. They also reported that the acidified vanillin solution is used for Colorimetric determination of Azadirachtin-related limonoids [6].

Atmakuru Ramesh *et al*., have described the rapid preconcentration method for the determination of azadirachtin-A and -B, nimbin and salannin in neem oil samples by using graphitized carbon solid phase extraction. They have just defatted the neem oil sample and extracted by mixing it with hexane and hexane solution is made to pass through a graphitized carbon black column. The azadirachtin-A and -B, nimbin and salannin were eluted with 5 ml of acetonitrile and was quantified using HPLC with UV detection. They reported the recovery percentage of azadirachtin-A and -B, nimbin and salannin in fortified oil samples were 97.4–104.7% respectively. There is no interferences during the HPLC analysis of the neem oil sample after preconcentration using graphitized carbon black [7].

Moacir Rossi Forim *et al*., have quantified the Azadirachtin and 3-tigloylazadirachtol in Brazilian seeds of Azadirachta Indica using a rapid, sensitive and selective HPLC-UV and HPLC-MS/MS method. For HPLC-UV the mobile phase used was MeOH: acetonitrile: THF: H_2O (36.75:7.35:4.9: 51) and the maximum absorbance was found at 217nm. Mobile phase consisted for HPLC-MS/MS was an isocratic mixture of acetonitrile: MeOH: THF: H_2O (34:4:1: 61) at flow rate of 0.4 mL min^{-1}[8].

Sara R. Fernandes *et al*., have analysed the extraction of Azadirachtin by using separating HPLC technique. After extracting the Azadirachtin from the seeds, fruits and etc. The Azadirachtin was detected in the range of 214-220nm [9].

NishaDahiya *et al*., have done the characterization and isolation of the neem to get Azadirachtin. By carrying out the NMR and Mass analysis they have found one oftheir analyzed fractions as mixture of Azadirachtin A and B. After that purification is done by HPLC using the mobile phase n-hexane: Ethylacetate (1:1) at 217nm [10].

OtmarSchaaf *et al*., have reported work on Rapid and sensitive analysis of Azadirachtin and related triterpenoids from Neem by high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. Analysis is done by HPLC using the mobile phase acetonitrile:0.1% trifluoroacetic acid (80:20) at 217nm [11].

O.J. Pozo *et al*., have developed a rapid and sensitive LC–ESI-MS–MS method for the determination of azadirachtin and abamectin residues in orange samples. Acetonitrile was used to extract the samples in a high-speed blender. An aliquot of the extract was immediately injected into the LC-ESI-MS-MS apparatus following the addition of sodium acetate. Under MS-MS settings, the technique 1's greatest sensitivity was attained utilizing [M1Na] adducts as precursor ions. With strong reproducibility (10%), recoveries for both drugs from spiked orange samples at 0.01 and 0.1 mg/kg were above 80%. From a regulatory perspective, the method detection limits attained were sufficient for determining these pesticides in this type of sample [12].

Deepak Sharma *et al*., have reported Development and Validation of Stability Indicating UV-Visible Spectrophotometric Method for Simultaneous Estimation of Neem (Azadirachtin) and Curcumin in Pharmaceutical Tablet Dosage Form. To simultaneously estimate azadirachtin and curcumin complex in combined pharmaceutical tablet dose form, a stability indicating UV Visible spectrophotometric method must be developed and validated by combination at two wavelengths, 271 nm and 421 nm. In the concentration range of 50–450 g/ml for azadirachtin and 200–1000 g/ml for curcumin complex, the technique adhered to Beer Lambert's law. The calibration curve's good linearity was confirmed by the high correlation coefficient (R2) values. Following the forced degradation studies, there were no appreciable changes in absorbance.

Azadirachtin and the curcumin complex were discovered to be 98.80 + 0.92 and 95.80 + 0.00 years old, respectively. The routine invitro dissolution analysis of the azadirachtin and curcumin complex in its combined pharmaceutical tablet formulations can be successfully conducted using the proposed method [13].

Steven V Ley *et al.,* havereported "Conxmtivist" Approach to Organic Structure Determination Lsd-Programme Assisted NMR Analysis of the infect Antifeedant Azadirachtin. The main instrument used for NMR investigations was a Bruker AC300 spectrometer that had been modified for inverse detection and was outfitted with a BFX-5 heteronuclear decoupler and an inverse configuration probehead. The azadirachtin 5OOMHz IH NMR data. Unless otherwise noted, samples typically contained 15 mg of the chemical in 0.5 ml of deuteriochloroform, and all data were taken at 27 °C. Rapid column chromatography on silica gel was used to isolate pure azadirachtin from a crude sample (309) of Neem seed extract employing a 7:3 ratio of ethyl acetate: petroleum ether as the solvent system [14].

Hui-p1ng huang *et al*., have reported Analysis of azadirachtin by supercritical-fluid chromatography. It is simple to use packed column supercritical-fluid chromatography to quantitatively determine the natural insecticide azadirachtin in crude neem seed extract, with detection by UV absorption at 210–220 nm and utilizing carbon dioxide: methanol as the mobile phase. 10 ng at 212 nm was the smallest amount of azadirachtin that could be detected [15].

R. Thejavathi *et al*., have reported Determination of azadirachtin by reversed-phase highperformance liquid chromatography using anisole as internal standard. Using anisole as the internal standard, a precise and practical approach for quantitating the well-known biopesticide azadirachtin by reversed-phase HPLC has been devised. In the concentration range of 0.00002% (w/v) to 0.004% (w/v) of azadirachtin in solution (0.2-40 ppm) or 2-400 ng by weight of azadirachtin (10/xl injection; 20 ng of internal standard), the procedure is precise and accurate to within 1.0%. The chemical is reasonably stable (95-99%) in a variety of common solvents at room temperature (7 days) and to ultrasound (30 min), according to application of the approach to stability studies of azadirachtin. The development of an effective sample preparation process has resulted in a recovery of 99.5% - 4% [16].

María Nella Gai *et al*., have reported An HPLC Method for Determination of Azadirachtin Residues in Bovine Muscle. It has been created to use high-performance liquid chromatography (HPLC) to measure the presence of azadirachtin (A and B) residues in beef muscle. A neutral triterpene known as azadirachtin is a chemotherapeutic medication that works well to eliminate several nuisance insects in horses, stables, horns, and fruit. UV detection and isocratic elution are used in the real HPLC procedure. The biological matrix was purified by solid-phase purification and liquid-liquid extraction. With a C18 analytical column, a water: acetonitrile mixture (27.5:72.5, v/v) as the mobile phase, 1 mL/min as the flow rate, 45°C as the column temperature, and a UV detector at 215 nm[17].

J. D. Warthen Jr *et al*., have reported Estimation of Azadirachtin Content in Neem Extracts and Formulations. A high-performance liquid chromatographic reversed-phase procedure has been developed whereby azadirachtin content can be estimated in crude extracts of neem and in dust formulations of neem. An estimation of the azadirachtin content is achieved through the use of an external azadirachtin standard and valley to-valley integration. Since azadirachtin seems to be the most patent insect feeding deterrent in these extracts and formulations, its content is a measurement of potency and represents an attempt at standardization at 217nm [18].

K.M.S. Sundaram *et al*., have reported High performance liquid chromatographic method for the analysis of azadirachtin in two commercial formulations and neem oil. It is reported that azadirachtin (AZ) content in neem formulations and neem oil can be determined using an enhanced high performance liquid chromatographic technique. Hexane and dichloromethane were used to extract sample aqueous methanol solutions. After being dried, the dichloromethane layer containing the AZ was reconstitute in ethyl acetate. A reversed-phase Spherisorb C-18 ODS column and an acetonitrile/water gradient system were used for separation. UV detection at 210 nm was used to keep an eye on the effluent. The analysis of AZ in neem formulations and neem oil was found to benefit from the method's speed and reproducibility. The limits of quantification and detection were 6 g/g and 3 g/g, respectively [19].

K. M. S. Sundaram *et al*., have reported High Performance Liquid Chromatographic Determination of Azadirachtin in Conifer and Deciduous Foliage, Forest Soils, Leaf Litter and Stream Water. The measurement of azadirachtin (AZ-A) in conifer and deciduous foliage, forest soil, litter, and stream water are detailed using a high-performance liquid chromatographic (HPLC) approach. Aqueous methanol was used to extract AZ-A from the solid matrices, after which it was concentrated, partitioned with hexane, and then again extracted into a layer of dichloromethaneand then analysed using a reversed-phase C-18 column with UV detection at 210 nm and an acetonitrile/water gradient system. Only the procedures following the extraction of dichloromethane were necessary for the analysis of AZ-A in the stream water. For soil and foliage, the limits of detection were 0.2 µg/ml, 0.25 µg/ml, and 5.0 µg/ml, respectively. average total mean recoveries from fortified terrestrial samples. With strong repeatability, the overall mean recoveries from terrestrial samples fortified with AZ-A at concentrations ranging from 0.50 µg/ml, to 10.0 µg/ml, and from 10.0 µg/ml, to 200 µg/ml, in stream water were > 80%. By examining the recovery levels of AZ-A in samples supplemented with regular Margosan-0, the method's applicability was demonstrated [20].

Reza Farjaminezhad *et al*., have reported Establishment of green analytical method for ultrasound-assisted extraction of azadirachtin, mevalonic acid and squalene from cell suspension culture of Azadirachta indica using response surface methodology. In this study, azadirachtin, mevalonic acid, and squalene extraction from A. indica cell suspension culture was optimized using response surface approach. The Box-Behnken Design was applied to the factor optimization. HPLC-DAD identified the azadirachtin, mevalonic acid, and squalene. Results indicated that for target chemicals from cell suspension culture of A. indica, the best conditions (solvent, temperature, and ultrasonication time) were as follows. Azadirachtin was produced in water at a temperature of 35 °C and an ultrasonication time of 20 min, yielding 86.445 mg/g DW; mevalonic acid was produced in 50% ethanol at a temperature of 45 °C and an ultrasonication time of 30 min, yielding 33.671 mg/g DW; and squalene was produced in 50% ethanol at a temperature of 55 °C and an ultrasonication time of 10 min [21].

Joelma Abadia Marciano de Paula *et al*., have reported Ultrasound-assisted extraction of azadirachtin from dried entire fruits of Azadirachta indica A. Juss. (Meliaceae) and its determination by a validated HPLC-PDA method. Acetonitrile: water (40:60), a flow rate of 1.0 mL min-1, detection at 214 nm, and a C18 column (250 x 4.6 mm, 5 m) were used for isocratic reversed-phase chromatography. Brazilian law and ICH recommendations were used to identify the key validation factors. The findings showed that ethanol concentrations between 75 and 80% (w/w), a temperature of 30°C, and a material-to-solvent ratio of 0.55 g mL-1 were the parameters that produced the best UAE conditions. The analytical HPLC-PDA approach was shown to be straightforward, linear, precise, accurate, and robust [22].

Mohibb e azam *et al*., have reported Estimation of Azadirachtin-A Content of Emulsifiable and Solution Concentrates of Neem. A column chromatographic cleanup technique was created to get rid of the various surfactants and aliphatic and aromatic solvent components included in prepared neem. The silica gel (60-120 British Standard Sieve) and 250 mL of hexane-acetone (6 :4) were placed in a glass column. Fractional elution was used to eliminate contaminants that were obstructing the azadirachtin-A peak's area. More than 81% of azadirachtin-A was recovered [23].

Penmatsatanuja *et al*., have reported Development and Evaluation of Thin-Layer Chromatography-Digital Image-Based Analysis for the Quantitation of the Botanical Pesticide Azadirachtin in Agricultural Matrixes and Commercial Formulations: Comparison with ELISA. Analyzing azadirachtin in processed commercial pesticide formulations and spiked food matrixes while utilizing acidified vanillin reagent as a post chromatographic derivatizing agent helped to confirm the procedure. It was easy to recognize the separated azadirachtin as a green patch. The R_f value, which was discovered to be 0.55, was comparable to a reference standard. Based on the linear regression analysis, $r^2 = 0.996$; $y = 371.43 + (634.82)\ x$, a standard calibration plot was created using a reference standard. The method was found to be 0.875g azadirachtin sensitive. Azidirachtin recovered between 67-92% in spiking tests at the 1 ppm (µg/g) level in a variety of agricultural matrixes, including brinjal, tomato, coffee, and cotton seeds. The approach found that the azadirachtin content of commercial neem formulations ranged from 190 to 1825 ppm (µg/mL). The current method was also contrasted with an immunoanalytical technique called an enzyme-linked immunosorbent test that was created earlier in our lab.

Fischer's F-test statistical comparison of the two procedures revealed no statistically significant variance difference, indicating that both methods are comparable [24].

Pierluigicaboni *et al.,* have reported Persistence of Azadirachtin Residues on Olives after Field Treatment. The acetonitrile/water gradient elution method that was used enabled azadirachtin to be separated chromatographically. The azadirachtin retention time at the chromatographic conditions mentioned above was 11.3 min, while the run time was 15 min. As soon as the extraction was done after sample, there were no interference peaks in the olive chromatograms [25].

Jianming Dai *et al*., have reported Extraction and Colorimetric Determination of Azadirachtin-Related Limonoids in Neem Seed Kernel. The best circumstances for carrying out the assay were chosen through the examination of numerous elements impacting the sensitivity of detection, including the concentration of vanillin, acid, and the time needed for color production. An excellent linear relationship between the absorbance at 577 nm and the standard azadirachtin solution concentration in the range of 0.01-0.10 mg/mL was discovered under ideal conditions. The vanillin assay was also used to evaluate various extraction methods. When the extracts were extracted in methanol and then partitioned in dichloromethane, the HPLC analysis of the extracts revealed that about 50% of the value derived by the vanillin assay represented azadirachtin content [26].

P. T. Deota *et al*., have reported estimation and isolation of azadirachtin-a from neem [azadirachta indica a. Juss] seed kernels using high performance liquid chromatography. On a reverse phase C18, analytical HPLC-UV was performed using a solvent solution of methanol and water (60:40 v/v), which was run isocratically at a flow rate of 1 mL/min and an average pressure of 1500–2000 psi for an injection volume of 20 L. At 217 nm, the peak matching to Aza-A was found. In the chromatographic process, 30 minutes passed. At a flow rate of 15 mL/min and column pressure of 400–500 psi, the peak was discovered at 217 nm. Aza -A peak appeared under these circumstances at 9.16 minutes during the preliminary run. Fraction "A" represents the amount of eluent collected when the Aza-A peak ascended from the baseline. Fraction "B" represents the amount of eluent collected when the peak sank back to the baseline. Analytical HPLC-UV confirmed the Aza-presence A's in fractions "A" and "B." By eluting the column with pure methanol for 10 to 15 minutes at a flow rate of 20 mL/min, the less polar chemicals could be eliminated. For another 20 to 25 minutes, the column was restabilized with methanol:water (60:40 v/v) solution[27].

J.K. Lalla *et al*., have used Azadirachtin as a Biomarker Compound in HPTLC Assay of Seed and Seed Oil of Azadirachta indica A. Juss. The reference standard azadirachtin was employed in this HPTLC investigation as a marker compound for the quantitative quantification of neem oil and powdered seed. Toluene-ethyl acetate-formic acid, 5 + 4 + 0.5 (v/v), was one of the mobile phases tested that provided high resolution of azadirachtin at RF 0.4. Following plate development, the spots were detected via derivatization with vanillin-sulfuric acid reagent, and their intensity was gauged at a wavelength of 667 nm. These experiments established the azadirachtin's identification and demonstrated that the azadirachtin standard's chromatographic purity was 98%. Using a common azadirachtin, the method's many characteristics were evaluated for validity. It was discovered that the LOQ was 100 ng and the LOD was 40 ng. In the concentration range of 100 to 1000 ng, a linear relationship between the response and the amount of azadirachtin was discovered; the correlation equation was $y = 0.104x - 1.3613$ and the correlation value was 0.998. The precision discovered by analysis of the standard was in the RSD range of 0.71 to 0.98, demonstrating the accuracy of the approach. The accuracy for the standard was determined to be between 98.14 and 100.98%. The approach was found to be precise since the azadirachtin standard spectrum and the azadirachtin isolated from samples of neem seed and neem oil (all at RF 0.40) were totally superimposable and showed no interference from analogues that were closely related to the test substance. Following the addition of neem seed and neem oil, the mean percentages of azadirachtin recovery were 92.73% and 94.56%, respectively. By using this HPTLC approach, the azadirachtin content of neem oil and seed was calculated to be 0.088 mg g-1 and 2.3 mg g-1, respectively [28].

Priyanka Srivastava *et al.,* have reported the Increased production of azadirachtin from an improved method of androgenic cultures of a medicinal tree Azadirachta indica A. Juss. The azadirachtin, a tetranortriterpenoid, demonstrated HPLC elution at 6.39 min in the standard and sample extracts that were made using the materials and techniques methodology. With a correlation coefficient (R2) of 0.9889, the calibration curve for the azadirachtin standard demonstrated satisfactory linearity at the tested doses (0.0625 mg/ml to 1 mg/ml). The

amount of chemicals present in the crude sample was determined using the equation created from the curve by an external standard method. The standard samples at the same concentration were examined for precision at least five times in the same day, yielding an RSD value of 3.99%. Similarly, the value for intraday variability was 3.78 percent when the same concentration of the standard compound was run three times with one day in between each run [29].

Nutan Kaushik *et al*., havedetermined the azadirachtin and fatty acid methyl esters of Azadirachta indica seeds by HPLC and GLC. A quick and affordable technique has been created to gauge the fatty acid composition and azadirachtin content of neem kernels. Crushed neem kernels are steeped in ethanol for an entire night. The resulting extract is filtered through a 0.22 m membrane before being analysed by HPLC. Acetonitrile-water (40:60) 1 mL min-1 is used as the mobile phase on an RP-18 column to separate the peaks, which are then seen at 214 nm. The fatty acids in the kernel powder are directly transmethylated for one hour in a water bath while being heated with methanol, acetyl chloride, and benzene (20:1:4, v/v). The resulting fatty acid methyl esters (FAMEs) are extracted in hexane and subjected to GLC analysis. Using a RH-Wax column and temperature programming (170-200 °C at 2 ° min-1), the FAMEs are separated. Utilizing a FID, the peaks are found. Both techniques don't need the seeds to be cleaned up or defatted. As a result, sample preparation is quicker, simpler, and more affordable [30].

De menezes *et al*., have reported determination of biopesticide azadirachtin in samples of fish and in samples of water of fish ponds, using chromatography liquid of high performance. The creation of a new analysis method for the extraction and determination of the biopesticide azadirachtin present in actual fish samples and samples of fish pond water allows the injection of fish extracts and fish pond water in a column chromatographic ISRP-C18 without the need for prior sample preparation. With a lower standard deviation of 14.6%, the azadirachtin recovery rates in fortified fish samples were higher than 90%. Azadirachtin's detection and quantification limits were discovered to be 0.02 and 0.12 micro gm/L, respectively. Azadirachtin was found in masses ranging from 0.14 to 2.317 micro gm/L in 86% of the fish samples tested (42 samples) [31].

Conclusion:

Quality control is one of the very important and essential steps in the manufacturing of herbal preparations as quality of product affects the safety and efficacy of medicines. Quality control is mainly applied for both raw materials along with excipients used and finished product. Many herbal medicines and their formulations containing Azadirachtin are available inmarket. Spectroscopic and Chromatographic methods play great role in the quality control and standardization of Azadirachtin in its isolated form, extract or any other herbal or polyherbal preparation.

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