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QBD APPROACH IN GACP FOR THE CONTROL OF AFLATOXINS IN COLLECTION AND STORAGE OF ABELMOSCHUS MOSCHATUS SEEDS & TO IDENTIFY AFLATOXINS USING HPLC-FLD TECHNIQUE

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Keywords:

QBD, GACP, *Abelmoschus moschatus*, Aflatoxin, HPLC-FLD, QuEChERS

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ABSTRACT: The Cultivation of *Abelmoschus moschatus* plants for collecting seeds used for medicinal purposes is affected by Mycotoxins which are substances that moulds create under particular environmental circumstances. These pollutants harm both human and animal health. Mycotoxins are difficult to get rid of and are occasionally impossible. The best control is prevention. Controlling the moisture levels in herbal materials and storage conditions like temperature and/or relative humidity can help avoid the growth of mycotoxins. The different parameters like Harvesting season, Dry grain storage conditions, and crop rotation techniques affect the growth of the mycotoxins. In this trial, the plants are grown using different parameters affecting the production, the packaging material and storage conditions are also varied as per QBD approach and the level of mycotoxins, specifically aflatoxins (G_1, G_2, B_1 & B_2) are identified, quantified, and monitored. The Aflatoxins are identified and analyzed using the HPLC-FLD technique, a sophisticated method for estimating the aflatoxin content in ppb levels; the method is validated and provides accurate and precise results in the ppb levels. The Cultivation procedure, packaging and storage conditions and the method used for analysis provides good practices to avoid Aflatoxin content in *Abelmoschus moschatus* seeds.

INTRODUCTION: Herbal medicines play a pivotal part in health care, and over 75-80% of the world's population rely mostly on traditional or alternative medical systems for preliminary health care. Worldwide, *Abelmoschus moschatus*, often known as musk okra and a member of the Malvaceae family, is used historically to cure various medical conditions.

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The plant's biological properties and medicinal potential have been the subject of intense research by numerous scientists [1], and Many cultures believe it to be a panacea for all diseases. For gonorrhoea, the leaves are utilized; for renal problems and as a diuretic and antispasmodic, the roots are used.

To treat stomach issues, seeds are eaten in Egypt. Infusions, decoctions, or tinctures made from seeds are used to treat nervous debility, hysteria, and other nervous illnesses [2, 3]. Research in the fields of pharmacognosy and pharmacology has uncovered important details about medicinal plants' availability, botanical characteristics, growth methods, collecting, preservation, commerce and

therapeutic applications. All of these have aided in their adoption in contemporary medicine and inclusion in the pharmacopoeias of developed countries. Their availability from natural sources has decreased due to ever-increasing land use for food crops, destruction of forests, and concurrent indiscriminate exploitation of these products. On the other hand, the demand for both domestic consumption and export has been rising, necessitating the large-scale production of these crops. These crops must be incorporated into the nation's cropping systems to satisfy industry demand and support the upkeep of chemical composition, potency, and quality standards. The World Health Organization (WHO) has produced a pattern of good agriculture and field collection practices (GACPs) for medicinal plants, and the National Medicinal Plants Board (NMPB) and Department of AYUSH have jointly developed recommendations on GAPs for India. Establishing effective agronomic and gathering practices for herbal raw materials is required to ensure the acceptable and consistent quality of medicinal plants and herbal substances 1 (GACP) [4-8].

Ethnobotanical Information: *Abelmoschus moschatus* Medic, a Plant. Musk Mallow, a Common Name Malvaceae Family.

Authoritative Status:
Continent: Plantae (plants) Magnoliopsida (flowering agent)

Division: Malave's

Order: Malvaceae Family

Species: Moschatus

Genus: Abelmoschus

Habitat: For its seeds, which are widely employed for isolating aroma constituents, the tropical areas of Asia, Africa and South America [2]. It grows wild in India's hilly Deccan and Karnataka areas, as well as near the foothills of the Himalayas [9].

FIG. 1 & 2: WHOLE PLANT OF *ABELMOSCHUS MOSCHATUS* & SEEDS

A. moschatus whole plant extracts revealed the presence of lipids, proteins, fixed oils, flavonoids, sterols, tannins and phenolic substances [10]. *A. moschatus* seed contains 1315 g of fatty oil and 0.20.6% of essential oil per 100 g. Palmitic acid (20%), oleic acid (2025%), linoleic acid (5057%), stearic acid (2.54%), and minor amounts of myristic acid and palmitoleic acid are the primary components of the fatty oil. When ambrette seeds are crushed prior to steam distillation, odourless palmitic acid and aromatic components are distilled over, producing crude oil with a paste-like consistency. The aromatic components are concentrated on the seed's outer layer and distilling the entire seed yields a liquid essential oil with very little fatty oil [11, 12].

Cultivational Practices:
Traditional Versus GACP Methods:
Soil: Ambrette is a resilient plant that grows well in a variety of soil types. It appears on sandy loam soils with a pH of 7.0 quite effectively. It may produce well on soils with a pH range of 6.0 to 8.6. majorly, it favours loosened, fertile soils with good drainage. In India, the crop is widely grown up to an elevation of 1000 m under various climatic conditions. It is said to happen in all of India's drier regions. It can be produced twice a year in

Karnataka as an irrigated crop, once in June–July and again in October–November. While in Punjab and the Tarai region of Kuamon (Uttar Pradesh), the crop is grown during the wet season.

The ground should be deeply excavated and properly worked to achieve a fine tilth before planting the seeds. Given that the plant is a heavy feeder, it should be properly manured. Seeds can be sowed twice a year in the months of June through July and September through October. The germination of the seeds takes roughly 8 to 10 days. When sowing, 2-4 seeds may be buried one centimeter deep per hill. The germination of seeds is improved by pre-soaking them in water for 24 hours. Around 6 kg of seeds are required for sowing a hectare of land.

Harvesting and Yield: After seeding for approximately 2 1/2 months, the crop begins to flower. The blossoms produce their fruits in around 3–4 days. From planting to fruit maturity, the fruit needs roughly two months. When the pods become blackish and white stripes start to develop at the angles of the fruit's ridges, they are ready for harvest.

A lengthy blossoming season makes harvesting challenges. The fruit should be carefully picked because it has stiff hairs that itch the skin. Depending on the availability of mature, ripe fruits, it must be done frequently at intervals of 7 to 10 days. It is a 170-180 duration crop and 20–25 plucking operations must be completed. The pods must then be shade dried [13].

The conventional practices are generally more prone to Aflatoxin contamination as they are cultivated, collected and stored in an environment suitable for the fungus's growth and lead to aflatoxin contamination due to improper harvesting methods, unhygienic collection, and storage conditions.

Prevention and Control: Getting rid of mycotoxins can be difficult or impossible. Prevention is the finest form of control. Mycotoxins can be avoided by lowering the moisture content in food goods and managing storage factors like temperature and/or humidity. Most moulds are found in soil and air naturally. Agricultural products can be contaminated by mould, although the development and toxin generation of the mould can be influenced. Mould development and mycotoxin production are often prevented by lowering the moisture level to the equivalent of less than 0.70 water activity (about 14.5% moisture by weight).

To Reduce Mycotoxin Contamination, the Canadian Food Inspection Agency (CFIA) Suggests the following Management Techniques: Limit damage from insects and birds. (Damaged kernels are prone to mould contamination).

Grain should be harvested as soon as possible to prevent high moisture levels. (High moisture environments are favorable for the growth of mould). Dried grain (Low moisture conditions inhibit mycotoxin development after harvest).

Maintain anaerobic conditions in the silo to prevent the growth of mould and mycotoxin contamination. (Mould cannot develop in truly anaerobic environments.) Use crop rotation to reduce the spread of mould from one year to the next. Avoid growing crops near fields where the disease can move from one crop to another if they are susceptible to mould infestation [15].

Detection and Estimation of Mycotoxins: There were found to be 18 different kinds of aflatoxins. The key constituents of aflatoxin are B_1, B_2, G_1, and G_2. Milk may contain aflatoxins M_1 and M_2, while other aflatoxins may not be present. Aflatoxin B_1 (AFB1) is commonly found in food products and cultures (the most dangerous type of Afs). Very sensitive isolation and analytical procedures are used in analytical methods (TLC, HPLC-FLD & MS, QDA detector), To identify mycotoxigenic fungi in variety of, herbal materials, human foods, and feeds to animals, immunological techniques such as the Radio Immuno Assay (RIA), Enzyme Immuno Assay (EIA), or Enzyme-Linked Immuno Sorbent Assay (ELISA), as well as the identification of mycotoxigenic moulds by molecular biological techniques are more expedient and objective methods.

Aspects of Mycotoxins Include: Aflatoxins break down at their melting points, which range from 237°C to 260°C (G1) and 299°C (M_1),

- ✓ The creation of cultivars resistant to diseases using GACP approaches such as: Agro-technical procedures, such as crop rotation, soil tillage, fertilization, variety selection, and plant protection measures (diseases, pests, weeds, *etc*).
- ✓ Ensure suitable conditions for storage [14].

QBD Approach in GACP: The pharmaceutical manufacturing industry has adopted and used quality by design (QbD) [16]. Critical material attributes (CMAs), critical process parameters (CPPs), and critical quality attributes (CQAs) are related in the application of quality by design (QbD), and the design space is constructed based on the permissible ranges of CQAs [17]. Herbal medications and chemical drugs differ in two ways, affecting how their QbD is implemented. First off all, when compared to chemical drugs, the quality variance of herbal raw materials is very great. Even herbs gathered in the same location but at a different period may have active chemicals with distinctly different contents from those found in medicinal plants from the same region. The second one is the variance in raw material quality.

The Materials can get Contaminated During the Process of:

Harvesting: Carried out from November to January (5-8 Harvests) as the moisture content will be favorable for the growth of the fungus.

Pre-harvesting: Remove the weeds and other plants if noticed in the field at the time of harvesting, as it leads to physical contamination.

Tip drying/ fruit drying may be considered for judging the maturity of the crop. When three-quarters of the body of the majority of pods have turned a dark blackish-brown and just started to open. The crop is ready for the first harvest at 120-140 days after sowing, the next harvest is carried out as and when the fruit dries (@ 15 – 20 days intervals)

At-harvesting: The farmers carry out common manual harvesting by handpicking the dried fruit. The pods/fruits should be spread on floor or tarpaulin sheets to dry for 1 or 2 days. Whole fruit or opened fruit are dried in the sun and stored.

Post-harvesting: The harvested fruits/ pods are spread over the threshing floor to dry. The fruits/ pods will dry and become brown colour. The fruits/ pods are threshed with a club/ rod/ wooden stick to open up the pods and separate the seeds. Seeds are winnowed, cleaned, and stored.

Packing & Labeling: Packed in whatever available packaging materials and stored together along with other plant harvests leading to cross-contamination of the material. The seeds are stored at a moisture content of 10 – 12 % for long term.

Considering the above problem, the first CQA's in this GACP practice can be considered as Collection practices during harvesting. Not mature seeds are collected, mature acropetally, so observation in the collection is a must, and harvest extends 3-4 months during winter, season; bad or unintentional practice of collecting raw materials using unconventional techniques leads to cross-contamination.

The second CQA is the moisture content in the materials leading to fungal growth or drying on roads, leaving it in open conditions and not covering the materials while drying, promoting the growth of mycotoxins.

Third CQA can be attributed to the storage conditions of the seeds during harvesting and post-harvesting because of the types of materials used for packing and storing the materials in different conditions for a longer time.

TABLE 1: THE MATERIALS WERE COLLECTED FROM DIFFERENT PARTS OF INDIA FOR THE STUDY AND WERE PACKED IN DIFFERENT PACKAGING SYSTEMS AND STORAGE CONDITIONS AS UNDER

State	Agro-climatic zone	season	Codes given	Woven sack	HDPE Containers	Polythene Bags	Gunny bags
Gujarat	Gujarat plain & hilly region	June to July	AMS/GJ (Series -1)	(Cond-A & B)	(Cond-A,B & C)	(Cond-A & B)	(Cond-A & B)
Andhra Pradesh	East coast plains and Hilly region	June to July	AMS/AP (Series -2)	(Cond-A & B)	(Cond-A,B & C)	(Cond-A & B)	(Cond-A & B)
Karnataka	Southern plateau and hilly region	June to July	AMS/KA (Series -3)	(Cond-A & B)	(Cond-A,B & C)	(Cond-A & B)	(Cond-A & B)

Condition A (Cond-A)	30 ^{0}C/65% RH ±5%
Condition B (Cond-B)	40 ^{0}C/ 75% RH ±5%
Condition C (Cond-C)	Cold condition 2-8 Degrees

Analysis of Aflatoxins

Methodology of Analysis: The HPLC method is developed and validated as follows:

Chemicals/Reagents Used: Methanol (HPLC Grade) is used as diluent, Potassium Bromide (AR Grade), Nitric acid (AR Grade), Acetonitrile (HPLC Grade), Sodium Chloride (AR Grade).

Standard Preparation: Aflatoxin mix standard should be allowed to come to room temperature; place 5 ml of methanol into a 10 ml volumetric flask.

Add 100 µl of undiluted standard mix to the volumetric flask and mix well. Make up the volume with methanol.

[Aflatoxin Standards are procured from R-Bio Pharma consisting of B_1 & G_1 (1.5 ppm/mL), B_2 & G_2 (0.3 ppm/mL)].

HPLC Condition:		
Column	:	C18 Luna ODS Phenomenex (250 X 4.6) mm ,5µ
Mobile Phase	:	Purified Water: Methanol: Acetonitrile (63:22:15) 350µl of dilute Nitric acid
Flow rate	:	1.0 ml/min
Injection Volume	:	20µL
Column temperature	:	25˚C
Detector (Fluorescent detector)	:	Excitation -365nm Emmission-435nm
External Derivatization	:	Ph-Red Cell/ Kobra Cell
Elution Order	:	G_2, G_1, B_2 & B_1

Note: The usage of Potassium Bromide is necessary if Ph-Red Cell is used.

Mobile Phase Preparation: Measure 630 ml of purified water and transfer to a 1000 ml volumetric flask. Similarly, measure 220 ml of methanol and 150 ml of acetonitrile and transfer them to the same volumetric flask. To this, add 50 µl of dilute nitric acid, mix gently. Filter the mobile phase through 0.45 µ membrane filter.

Extraction Solvent (Methanol: Water - 80: 20): Measure 800 ml of methanol and transfer into 1000 ml volumetric flask; to this, measure 200 ml of purified water and transfer into the same volumetric flask and mix solution gently.

Sample Preparation:

- Weigh about 25 g of uniformly powdered sample to be analyzed and transferred into a blender jar.
- Take 10g of blended powder into the QuEChERS kit (Code-26222, Make- Restek) + 5 grams of sodium chloride to render it to basic pH conditions.
- Add 30 ml of extraction solvent (Methanol: Water – 80:20). Cap the kit tube and shake it vigorously for 5 minutes and sonicate for 10 minutes.
- Centrifuge the contents at 5000 RPM for 5 mins and collect the clear supernatant layer.
- Pipette 10 ml of supernatant into 50 ml volumetric flask and make up the volume up to mark with purified water and mix well. This solution is used for passing through the Aflatest column.

Clean up Procedure: Aflatest-p (make-R-BioPharma) column should be attached to the pump stand. Drop by drop, drip 10 ml of filtrate (sample) through the column. After the column has processed the entire sample, rinse it twice with 10 ml of filtered water. Add 1 ml of methanol to the column and place an HPLC vial underneath the tip of the column. Fill the HPLC vial with the entire methanol eluent, then cap it. This sample is utilized for the HPLC system injection.

Procedure: Stabilize the instrument with the above-mentioned mobile phase, inject 20 µl of blank, standard six injections followed by sample solution into the column of the HPLC instrument and record the chromatogram for about 30 minutes.

System Suitability: Tailing factor not more than 2, Theoretical plate not less than 5000, Resolution not less than 2.0

Performed the Validation Parameters: The process by which the developer or user tests a method for dependability, accuracy, and precision of its intended purpose is known as method validation, and it is an essential component of method development [18]. To validate the methodological techniques in accordance with the current pharmaceutical regulatory requirements, a number of parameters must be investigated.

Specificity: By injecting diluent (blank), preparing the working standard and sample, and checking for interference during the analytes' retention times, the specificity is done. Peak purity index, peak asymmetry, theoretical plates, and resolution factor are used to determine specificity in **Tables 2** & **3.**

TABLE 2: SYSTEM SUITABILITY TESTING FOR METHOD

Name of the Analyte	Retention time (minutes)	Theoretical plates	Peak asymmetry	Resolution
G2	13.1	14778	0.75	-
G1	15.5	6263	0.82	3.9
B2	18.0	10677	0.95	3.4
B1	21.7	9070	0.75	4.6

TABLE 3: PEAK PURITY INDEX OF THE METHOD

Name of the Analyte	Standard		Sample	
	Peak purity index	Single point Threshold	Peak purity Index	Single point threshold
G_2	1	0.999	1	0.999
G_1	1	0.999	0.999	0.999
B_2	1	0.999	0.999	0.999
B_1	1	0.999	1	0.999

Repeatability: Five injections from a single standard preparation were used to establish the system's precision for the standard working solution. The average area under the curve (AUC) and relative standard deviation as a percentage (%RSD) were noted in **Table 4.**

TABLE 4: SYSTEM PRECISION OF THE METHOD

Injection no.	Aflatoxin G_2 (AUC)	Aflatoxin G_1 (AUC)	Aflatoxin B_2 (AUC)	Aflatoxin B_1 (AUC)
1	179039	333079	463459	604406
2	165000	331160	473022	629358
3	165846	322333	459065	633836
4	168229	324987	465679	604345
5	163316	324840	458507	604032
% RSD	3.72	1.40	1.27	2.19

Intermediate Precision: The intra-day precision of the method was established by injecting six injections from single preparation at the LOQ concentration respectively for B_1, G_1, B_2 & G_2 (established through 10 times the s/n ratio). The mean assay and % RSD values were calculated in **Table 5.**

TABLE 5: INTRADAY PRECISION OF METHOD

Injection No.	Aflatoxin G_2 (AUC)	Aflatoxin G_1 (AUC)	Aflatoxin B_2 (AUC)	Aflatoxin B_1 (AUC)
1	176744	325237	455511	603278
2	166012	331967	461381	629243
3	160209	322333	453212	637230
4	165310	319050	449994	632517
5	163316	313595	446839	606148
6	163182	319562	457928	625252
% RSD	3.46	1.94	1.17	2.28

Inter-day Precision: The inter-day precision of the method was established by injecting six injections from single preparation at the LOQ concentration respectively for B_1, G_1, B_2 & G_2 (established through 10 times the s/n ratio). The mean assay and % RSD values were calculated in **Table 6.**

TABLE 6: INTRA-DAY PRECISION OF METHOD

Injection No.	Aflatoxin G_2 (AUC)	Aflatoxin G_1 (AUC)	Aflatoxin B_2 (AUC)	Aflatoxin B_1 (AUC)
1	185255	351846	496111	673821
2	193102	337835	488788	650345
3	202857	334764	492616	670201
4	186284	337387	485577	664747
5	185458	337574	484577	650769
6	185785	334177	501429	645120
% RSD	3.72	1.92	1.32	1.81

The comparison of %RSD between intra and inter day precision was recorded.

Linearity: Linearity was performed by injecting eight different concentrations of the sample ranging from 5% to 150% of standard concentration (15 ppb of B_1 & G_1, 3 ppb of B_2 & G_2). The regression coefficient (R^2) was recorded and tabulated **Table 7.**

TABLE 7: LINEARITY OF METHOD

Name of the Aflatoxin	Regression equation	Regression coefficient (R^2)
G_2	52905x-1292.5	0.9996
G_1	30980x-2153.3	0.9991
B_2	143789x-811.42	0.9995
B_1	54573x-3231.2	0.9998

Accuracy: Accuracy can be defined as the exactness of an analytical method or the closeness of obtained results. Accuracy was derived by spiking the 100% concentration of the standard to matrix matched blank, and the recovery of the respective analyte is recorded in **Table 8.**

TABLE 8: RECOVERY OF THE METHOD

Injection No. (Spiked Concentration)	Aflatoxin G_2 (% Recovery)	Aflatoxin G_1 (% Recovery)	Aflatoxin B_2 (% Recovery)	Aflatoxin B_1 (% Recovery)
50%-1	101.41	82.12	96.40	77.08
50%-2	120.28	87.13	90.33	84.86
50%-3	102.64	80.77	95.24	84.54
100%-1	114.08	101.01	106.56	114.08
100%-2	110.99	93.13	96.52	104.10
100%-3	105.34	107.48	95.69	102.49
150%-1	101.95	98.15	97.80	90.35
150%-2	102.41	105.15	95.60	104.19
150%-3	112.46	104.00	103.32	107.54
Mean Recovery	107.95	95.33	97.49	96.58

Robustness: The method's reliability was assessed in light of intentional modifications to its parameters, such as variations in the mobile phase's flow rate (± 0.1 ml/min) and the excitation wavelengths (±3 nm), which will affect the RTs and responses of the analytes. The sample, standard, and diluent were analyzed for small changes in method parameters by injecting two replicates of single preparation, and readings are recorded in **Tables 9** & **10.**

TABLE 9: ROBUSTNESS OF THE METHOD

Robustness variables	Aflatoxin G_2 (%RSD)	Aflatoxin G_1 (%RSD)	Aflatoxin B_2 (%RSD)	Aflatoxin B_1 (%RSD)
Flow rate (0.9 ml/minute)	3.17	1.63	2.96	3.59
Flow rate (1.1 ml/minute)	2.31	7.21	4.37	4.64
Excitation -362nm & Emmission-435nm	2.85	2.60	2.83	2.30
Excitation -368nm & Emmission-435nm	2.34	1.11	2.87	3.21

TABLE 10: COMPARISON BETWEEN ASSAY OF ROBUSTNESS AND INTRADAY PRECISION

Robustness variables	%RSD	Comparison % RSD between assay of robustness and intra-day precision
Flow rate (0.9 ml/minute)	1.68	2.41
Flow rate (1.1 ml/minute)	2.62	1.79
Excitation -362nm & Emmission-435nm	0.12	0.52
Excitation -368nm & Emmission-435nm	0.39	0.81

RESULTS AND DISCUSSION: The Study performed elucidates the impact of GACP cultivation practices, the education or knowledge provided for farmers in every step of Harvesting, collection, processing, and storage conditions, on the presence and growth of aflatoxins in *A. moschatus* seeds. Good collection practices are being taught to farmers about when and how to collect the seeds, and Poly houses are used for drying the seeds as recommended by GACP. Avoid cross-contamination by using proper footwear and collection bags to avoid cross-contamination and moisture content.

The results from the three different conditions (Condition A, B & C) and in different packaging systems of Woven Sacks, poly bags, HDPE containers, and Gunny bags clearly show that the aflatoxins content, which was 11 to 25ppb in moisture content of 2.6 to 4.3 has raised to 11-33 ppb and 10 to 32ppb in woven sack bags (Graph-1 & 2) with a significant increase in the moisture content from 2.6 to 5.1%, aflatoxins content has raised to 11-33 ppb and 12 to 33ppb in poly bags (Graph-6 & 7) with a significant increase in the moisture content from 2.6 to 6.2%, aflatoxins content has raised to 19-46 ppb and 23 to 52 ppb in gunny bags (Graph-8 & 9) with a significant increase in the moisture content from 2.6 to 7.2% showing the effect of moisture and humidity is playing a vital role in the two different conditions (Condition-A & B respectively). The seeds stored in the HDPE containers at all three different conditions of temperature and humidity showed less or minimal growth of aflatoxins from 11-28 ppb, 11 to 30 ppb (Condition-A & B respectively) and a further increase in the growth of aflatoxins in the extreme condition from 12-33 ppb of (Condition-C) (Graph-3, 4 & 5)Based on the QBD study considering the moisture content, storage conditions and the packaging materials as CQA's, it is recommended to pack the *A. moschatus* seeds in HDPE or polybags and stored in an optimum condition of 30°C and 65% ±5% RH when storing for longer durations. Using secondhand bags and fertilizer bags should not be used as these may lead to cross contamination of materials and an increase in moisture content, which increases the chances of fungal growth and aflatoxin content.

The analytical method developed for estimation of the aflatoxin content using an HPLC-FLD detector showed good sensitivity and robustness, which was further validated, and the System suitability was executed by injecting a working standard mixture of Aflatoxin B_1, B_2, G_1 & G_2 in five replicates and the parameters like retention time, theoretical plates (N), peak asymmetry (As), & resolution (Rs) were studied **Table 2**. The results are found to comply with the acceptance criteria as per USP 621 general chapter. **Fig. 3** the observation recorded from validation are described below:

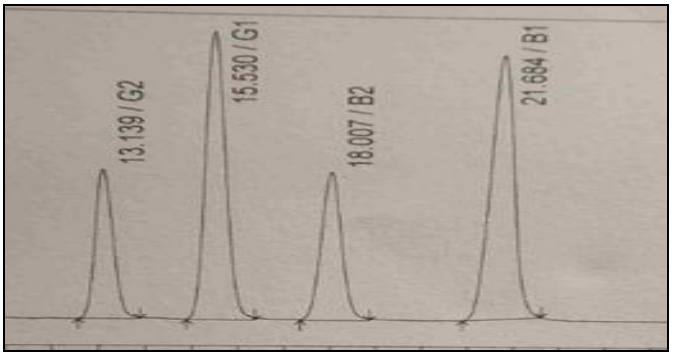


FIG. 3: CHROMATOGRAM SHOWING RETENTION TIMES OF B1, B2, G1 & G2 AFLATOXINS

Selectivity: (No interference with the peaks of interest due to mobile phase or diluent).

Precession: (G_2, G_1, B_2 & $B_1 \leq 10\%$ for 6 injections at LOD level).

Linearity: (G_2- 0.9998 G_1-0.9996, B_2-0.9995 & B_1-0.9991).

Accuracy: ($G_2$95.33% , G_1-107.95%, B_2-97.49% & B_1-96.58%).

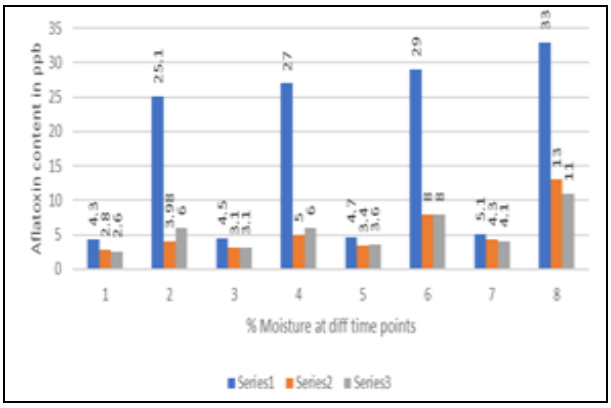
LOD: (G_2- 0.08ppb G_1-0.5ppb, B_2-0.1ppb & B_1-0.2ppb).

LOQ: (G_2- 0.24ppb G_1-1.5ppb, B_2-0.3ppb & B_1-0.6ppb). Robustness performed with a minor change in

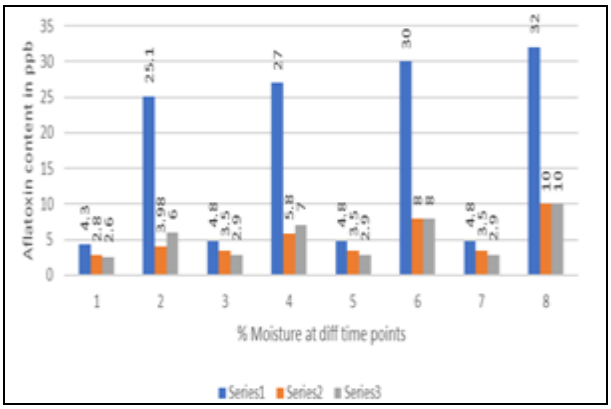
Flow Rate (±10%): At 0.9 ml/min: (G_2- 3.17% RSD G_1-1.63% RSD, B_2-2.96% RSD & B_1-3.59% RSD), at 1.1 ml/min: (G_2- 7.21% RSD G_1-2.31% RSD, B_2-4.64% RSD & B_1-4.37% RSD)

Excitation Wavelength Changes (±3nm): At 362nm: (G_2- 2.85% RSD G_1-2.60% RSD, B_2-2.83% RSD & B_1-2.30% RSD) at 368nm: (G_2- 2.34% RSD G_1-1.11% RSD, B_2-2.87% RSD & B_1-3.21% RSD)

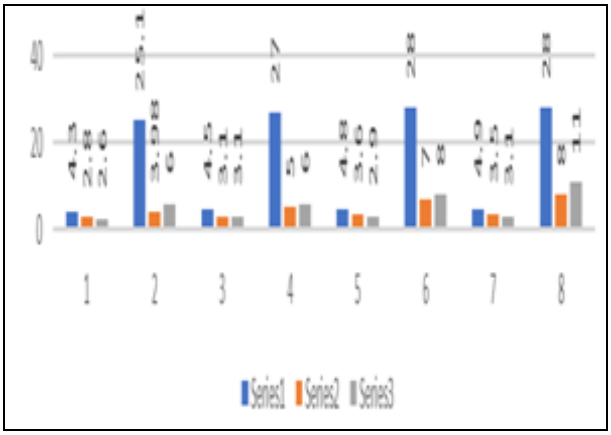
The Results show that the method is sensitive and robust enough to detect the Aflatoxins in PPB Level and Meet the Regulatory Requirements:



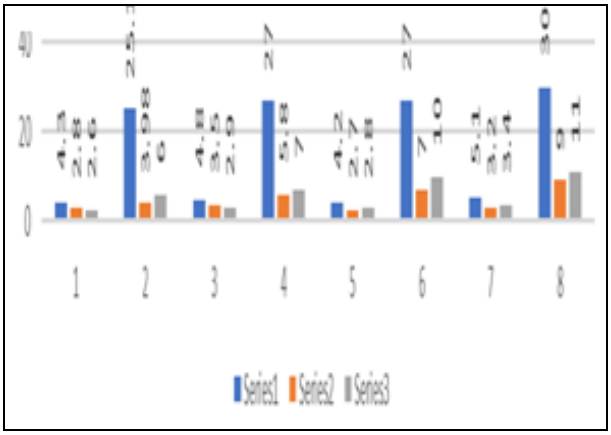
GRAPH 1: WOVEN SACK CONDITION A FOR 6 MONTHS



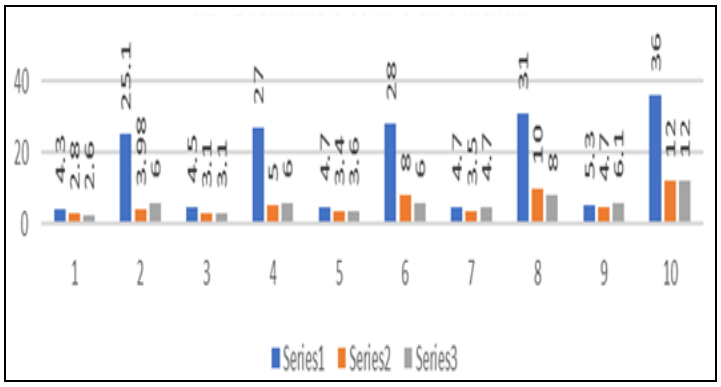
GRAPH 2: WOVEN SACK CONDITION B FOR 6 MONTHS



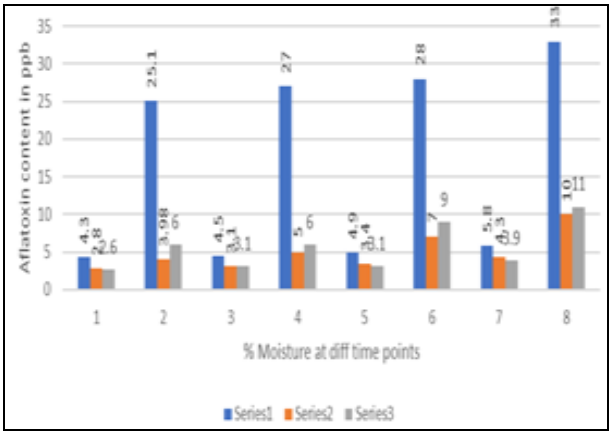
GRAPH 3: HDPE CONTAINERS COND-A TILL 6 MONTHS



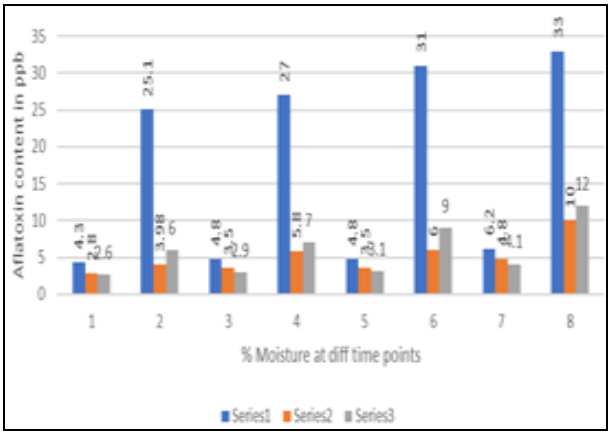
GRAPH 4: HDPE CONTAINERS COND-B TILL 6 MONTHS



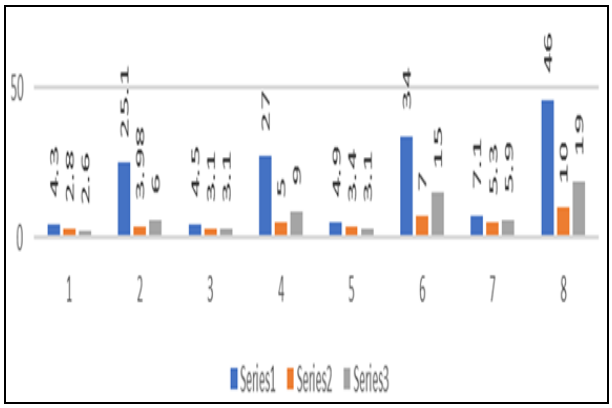
GRAPH 5: HDPE CONTAINERS COND-A TILL 6 MONTHS



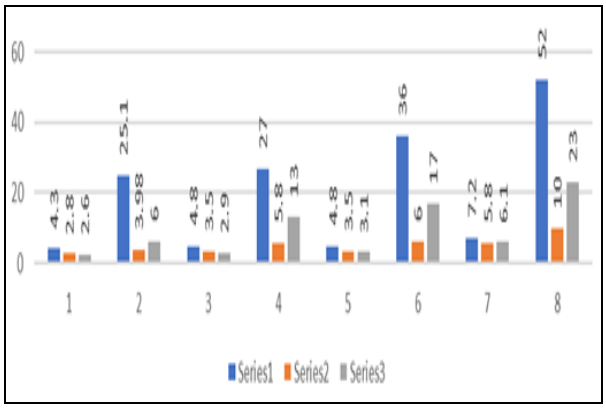
GRAPH 6: POLYBAGS CONDITION A FOR 6 MONTHS



GRAPH 7: POLYBAGS CONDITION B FOR 6 MONTHS



GRAPH 8: GUNNY BAGS COMPARISON OF COND-A TILL 6 MOTHS



GRAPH 9: GUNNY BAGS COMPARISON OF COND-A TILL 6 MOTHS

CONCLUSION: Implementation of an educated approach in every step of pre-harvesting, harvesting & post harvesting like crop rotation, collection, drying, and storage of the *A. moschatus* seeds using suitable packing and storage conditions like HDPE and Poly bags avoids contamination and moisture exposures preventing or reducing the presence and growth of aflatoxins in the seeds and if present, for the estimation of aflatoxins a straightforward, accurate, precise, robust and dependable HPLC-FLD approach can be applied. Utilizing retention times and UV spectral properties, the toxins were each separately classified against distinct, genuine, commercially available standards. According to the ICH Q2 R1 guidelines for the validation of analytical techniques, the method was fully validated, proving its applicability, repeatability, and linearity, and the results complied with the acceptance criteria of the regulatory bodies. This approach can be used in routine, stability-indicating analysis when toxicity analysis is required.

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CONFLICTS OF INTEREST: Nil

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