International Journal of Multidisciplinary Research and Development
Online ISSN: 2349-4182, Print ISSN: 2349-5979
Impact Factor: RJIF 5.72
Received: 04-10-2018; Accepted: 08-11-2018
www.allsubjectjournal.com
Volume 5 Issue 11; November 2018; Page No. 54-56

Comparative analysis of aflatoxin in the formulated nutraceutical supplement and market sample

Sasikala S[1], Dr. Radhaisri S[2], Deeptha Kumar[3]
[1] Research Scholar, Bharathiar University, Coimbatore, Tamil Nadu, India
[2] Associate Professor, Department of Food & Nutrition, PSG College of Arts and Science, Coimbatore, Tamil Nadu, India
[3] Research & Development Executive, Oriens Global Marketing Pvt Ltd, Chennai, Tamil Nadu, India

Abstract
Aflatoxins are natural toxins, produced by several fungal species and are secondary metabolites of mould fungi identified in toxigenic molds. The aflatoxin was assessed using the chromatography technique. The aim of the study is to estimate the formulated *GSMA* and to assess the aflatoxin content. The formulated Nutraceutical supplement was compared with market sample and FSSAI limit. The obtained results satisfied the limit and ensure the safety of the product.

Keywords: nutraceutical supplement, aflatoxin, FSSAI, market sample

Introduction
Aflatoxins are natural toxins, produced by several fungal species and are associated with morbidity or even mortality in animals, plants, and humans. These compounds have diverse chemical structures and a low molecular weight. Aflatoxins are often found in a large number of agricultural and food products throughout the world. In different stages such as the production, harvest, transport, and storage of agricultural products, aflatoxins can result in the contamination of human food or animal feed. Nevertheless, these fungi are not endemic to specific geographical areas or climates. In fact, fungal growth and toxin production occur only if the environment and conditions are suitable (Murphy *et al* 2006).
Aflatoxins (AFs), namely aflatoxins B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), are secondary metabolites produced by fungal species, such as Aspergillus flavus, Aspergillus parasiticus and Aspergillus nomius (Scaglioni *et al* 2014) [14]. AFs are carcinogenic, hepatotoxic, immune suppressive, genotoxic, antinutritional, teratogenic and mutagenic to humans (Partnan *et al* 2010, Sweeney *et al* 1999, Hussain and Barsel 2001) [1, 5, 9] and AFB1 was defined as a Group 1A carcinogen by the International Agency for Research on Cancer (IARC 2002) [2]. Due to the pernicious nature of AFs, many countries have established regulations to control the levels of AFs in food and agricultural products which are susceptible to fungal growth.
Improper storage, drought, and high humidity can lead to an increase in the growth of Aspergillus species in herbs and spices (Ozbey and Kabak 2012) [4]. Spices are mostly produced in humid and tropical countries, where a suitable environment for fungal growth is provided (Pesavento *et al* 2016) [6]. The FSSAI has determined the maximum tolerable limit for total aflatoxins.
The aim of this study was to investigate the prevalence of aflatoxin contamination in nutraceutical supplement and compare it with the market sample. Continuous assessment and control of fungal contamination in nutraceutical supplement can guarantee the health of consumers and promote competition in the international market.

Methodology
Analysis method for aflatoxin
Extraction
50 g of sample was transferred, 200 ml methanol was added followed by 50 ml 0.1N HCl, and blended for 3 min at high speed. Filtered the sample through what man no.1 paper or equivalent and collected 50 ml filtrate.

Partition
50 ml filtrate was transferred to 250-ml separatory funnel, 50 ml 10% NaCl solution added, swirled, 50 ml hexane was added, and shook gently for approximately 30 sec. Phases separated and drained lower aqueous layer into another 250-ml separatory funnel. The hexane layer was discarded. 25 ml CH2Cl2 was added and shaken moderately for 30 sec. Allowed phases to separate, and drained lower CH2Cl2 layer through 4-cm coarse granular anhydrous Na2SO4 in glass filter tube. The eluate was collected in the 250-ml beaker. Repeated partition with two additional 25-ml portions of CH2Cl2shaken vigorously and drained as above. The eluate was collected in a 250-ml beaker. The eluate was evaporated on the steam bath under a gentle stream of nitrogen to 2–3 ml (1–2 mm layer of eluate covered the bottom of the beaker).

Column chromatography
Slurry 2.0 g silica gel with approximately 10 ml ether-hexane (3 + 1) in 30-ml beaker; poured the slurry into the cleanup column, and washed beaker with additional 5 ml ether-hexane solvent to effect the transfer. Kept stopcock closed and let silica gel settle without tamping. Washed sides of the column with 2–3 ml ether-hexane (3 + 1), using a wash bottle. After gel settled, opened stopcock and while column drained, added

approximately 1 cm anhydrous granular Na 2SO4.Transferred extract from partition to the column. The beaker was washed with 0.5 ml CH2Cl2, using a wash bottle and collected wash in the column. To transfer the extract to the column, not more than 5-6ml of CH2Cl2 was used. With stopcock fully open, added 25 ml benzene-acetic acid (9 + 1); then added 30 ml ether-hexane (3 + 1) to the column, draining each wash to top of Na 2SO4. Washes were discarded. Aflatoxin was eluted with 100 ml CH2Cl2-acetone (9 + 1), and collected eluate in a 250-ml beaker. Evaporated solvent on the steam bath under a gentle stream of nitrogen to approximately 6 ml. And it was quantitatively transferred to 3-dram vial, using 2–3 ml CH2Cl2 as a wash. Eluate was evaporated almost to dryness on a steam bath in the aluminum block under a gentle stream of nitrogen. Remaining 200 ml was evaporated just to dryness under a gentle stream of nitrogen by holding vial in the palm of the hand and slowly rotating vial.

Derivatization

Added 200 µl hexane to the vial; add 50 µl trifluoroacetic acid (TFA) with Eppendorf pipette, cap vial, and vortexes vigorously for exactly 30 sec. This procedure was followed closely to ensure consistent reaction yields. Allowed mixture to stand for 5 min. Using Eppendorf pipette, added 1.950 ml H2O-CH3CN (9 + 1). Vigorously vortexes for exactly 30 sec.

HPLC determination

Using instrument parameters previously described in apparatus, successively injected 25 µl of derivatized standard solutions. Standard curve prepared to check the linearity of responses. Injected 25 µl TFA-treated sample solution (lower aqueous phase). If sample peaks were outside linear range, a diluted aliquot of TFA-treated sample solution to suitable volume with H2O-CH3CN (9 + 1), vortex, and injected another 25-µl portion.

Calculation

Used responses of a standard containing 500 mg B1 and G1 and 250 mg B2 and G2 for calculations.

$$\text{Aflatoxins, ng/g} = (Ps/Pst) \times C \times (2/10) \times 100 \times D,$$

Where Ps and Pst = peak areas or heights for sample and standard, respectively, per 25 µl injection; C = concentration of individual Aflatoxins in standard solution (0.5 or 0.25 ng/10.05 ml); and D = dilution factor if it is necessary to dilute final 2 ml injection solution

Results and discussion

The nutraceutical supplement, formulated *GSMA* supplement were selected for analysis of aflatoxin. The *GSMA* supplement was compared with the market sample. Both the market sample was estimated as per the FSSAI limit.

Aflatoxin level in formulated *GSMA* supplement

The results of formulated *GSMA* supplement for the presence of aflatoxin is given in Table 1.

Table 1: Comparison of aflatoxin growth with FSSAI safety limits

Aflatoxins	FSSAI Limits	Values
B1	30 µg/kg	7.4 µg/kg
B2	30 µg/kg	1.5 µg/kg
G1	30 µg/kg	1.0 µg/kg
G2	30 µg/kg	BLQ (LOQ: 0.5)

The aflatoxin content in the formulated *GSMA* Supplement was compared with FSSAI safety limits and all the values were found to be within the safety limits of FSSAI.

Comparison of aflatoxin with market sample

The results of formulated *GSMA* supplement for the comparison of aflatoxin with the market sample is given in Table 2.

Table 2: Comparison of aflatoxin growth with FSSAI safety limits

Aflatoxins	FSSAI Limits	*GSMA*	SAMPLE I
B1	30 µg/kg	7.4 µg/kg	20.5
B2	30 µg/kg	1.5 µg/kg	5.2
G1	30 µg/kg	1.0 µg/kg	3.4
G2	30 µg/kg	BLQ (LOQ: 0.5)	BLQ (LOQ: 0.5)

The aflatoxin content in the formulated *GSMA* Supplement and market sample was compared with FSSAI safety limits and all the values were found to be within the safety limits of FSSAI. In Market Sample I the values was little higher than the formulated supplement.

Conclusion

Aflatoxin contamination in food supplement, even at low levels, can be a serious threat, given the prevalent use in food preparation. Therefore, regular monitoring of food supplement is highly recommended, considering the popular use of food supplement around the world. Our study showed that the formulated *GSMA* aflatoxin level was under the FSSAI limit. It showed that the safety as well as the quality of the nutraceutical supplement and fit for consumer to consume.

Acknowledgment

The authors would like to express their deepest gratitude to P. Karthikeyan, CEO, S. Moulishankar, COO, S. Suman, CFO Oriens Global Marketing (P) Ltd, India for financial support for this study and permitting us to conduct this study. Our heartfelt thanks go to the Parthian Subramanian for his guidance and support.

Reference

1. Hussein HS, Brasel JM. Toxicity, metabolism, and impact of mycotoxins on humans. Toxicology. 2001; 167:101-134.
2. International Agency for Research on Cancer (IARC). Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, Monographs on the Evaluation of Carcinogenic Risks to Humans. World Health Organization, Lyon. 2002; 82:171-300.

3. Murphy PA, Hendrich S, Landgren C, Bryant CM. Food mycotoxins: an update. J Food Sci. 2006; 71(5):R51-65.
4. Ozbey F, Kabak B. Natural cooccurrence of aflatoxins and ochratoxin A in spices. Food Control. 2012; 28(2):354-61.
5. Partanen HA, *et al.* Aflatoxin B1 transfer and metabolism in human placenta. Toxicol Sci, 113, 216-225, doi: 10.1093/toxsci/kfp257 (2010).
6. Pesavento G, Ostuni M, Calonico C, Rossi S, Capei R, Lo Nostro A. Mycotic and aflatoxin contamination in Myristica fragrans seeds (nutmeg) and Capsicum annum, packaged in Italy and commercialized worldwide. J Prev Med Hyg. 2016; 57(2):E102-9.
7. Scaglioni PT, Becker-Algeri T, Drunkler D, Badiale-Furlong E. Aflatoxin B(1) and M(1) in milk. Anal Chim Acta, 829, 68-74, doi: 10.1016/j.aca.2014.04.036 (2014).
8. Sweeney MJ, Dobson ADW. Molecular biology of mycotoxin biosynthesis. FEMS Micro bio l Lett. 1999; 175:149-163.