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Determination of ochratoxin a in spices from Dharwad by high performance liquid chromatography

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ABSTRACT

Spices are important agriculture commodities because of their strong flavour, aroma and taste they are widely used to flavour the food preparations. Fungal contamination is one of the common problem in spice trade, due to poor agriculture and storage practices spices get contaminated from field to fork level, if they get conducive atmosphere for their growth they grow and produce toxic secondary metabolites called mycotoxins, mycotoxins are having toxic effects on Humans and animals. The most common mycotoxins are Aflatoxins, Ochratoxins, Fumonisins, Trichothecenes, Zearalenones and Ergot alkaloids etc. Among the mycotoxins Ochratoxins are most common toxins produced by mainly two genera of fungi, Aspergillus and Penicillium. The International Agency for Research on Cancer (IARC) classified Ochratoxin-A as possible Human carcinogen (Group 2B), were Ocharatoxin –A is common contaminant of various food stuffs including spices. The present study aims to investigate the concentration of Ochratoxin–A in selected spices from Dharwad. A total of 20 samples belonging to 5 spices were analysed for Ochratoxin-A content by HPLC (High Performance Liquid Chromatography) with fluorescent detector, after immunoaffinity column clean up, average percentage of recovery value (mean) is 87% , Limit of Quantification (LOQ) is 5.0 µg/Kg among the 20 samples 19 samples shows Ochratoxin- A content <5.0 µg/Kg but one black pepper sample shows Ochratoxin–A content 5.9 µg/Kg. Ochratoxin–A content of all the 20 analysed samples ranging from < 5.0 µg/Kg to 5.9 µg/Kg., so all the 20 analysed samples from Dharwad shows the Ochratoxin-A content within the EU consumption limit.

Key words: Spices, Mycotoxins, Ochratoxin-A HPLC and OTA

INTRODUCTION

Spices are strongly flavoured aromatic substances of vegetable origin they are commonly used to flavour the food preparations, fungi are predominant contaminants of spices but most of such mycopopulations are probably regarded as commensal residents on the plant that survived. Fungal contamination of spices is the one of the major problem in spice trade, if contaminated mycoflora gets conducive atmosphere for their growth, they grow and degrade the quality and taste of the spice. The potential for fungal spoilage and mycotoxin production is mainly depends upon the type of fungi present, composition of food, handling and storage conditions **[1]**. Mycotoxins are the secondary metabolites secreted by few species of fungi which are toxic to Humans and animals. The most common mycotoxins are Aflatoxins, Ochratoxins, Fumonisins, Trichothecenes, Zearalenones and Ergot alkaloids etc. There are many factors which influence the mycotoxin production Viz., Environmental conditions, storage conditions, fungal species, etc. **[2],** because of their toxic properties, health effects and economic losses mycotoxins drawn the attention of scientific community in recent years and also increased the awareness among the common people. Mycotoxins are having comparatively high thermostability even most of the food processing operations cannot destroy mycotoxin completely **[3].**

Ochratoxins are common mycotoxins produced by mainly two genera of fungi *Aspergillus* and *Penicillium.* **[4]** classified Ochratoxin-A as possible Human carcinogen (Group 2B) and also it has been described as nephrotoxic, teratogenic, immunotoxic and hepatotoxic to laboratory and domestic animals **[5]**, Based on slight chemical

structural differences they classified as Ochatoxin A, B and C were Ochratoxin-A is most toxic ochratoxin **[6].** Therefore this study aims to investigate prevalence level (concentration) of ochratoxin –A in selected spices from Dharwad. A total of 20 samples belongs to 5 spices were analysed for ochratoxin –A content by HPLC-FD after immunoaffinity column clean up.

MATERIALS AND METHODS

Sampling

A total of 20 samples belonging to 5 different spices viz., *Elettaria cardamomum*, Maton. (Cardamom), *Coriandrum sativum*, L. (Coriander), *Cuminum cyminum*, L. (Cumin), *Cinnamomum tamala*, T. Nees and C.H. Eberm (Indian cassia) and *Piper nigrum*, L. (Black pepper) 250 gram of each sample of unknown variety were randomly collected in polyethene bags from four different places from Dharwad and stored at 4^0 C until the analysis.

Extraction and quantification of Ochratoxin –A

Ochratoxin–A was extracted and analysed using **[7]** for extraction of ochratoxin -A 10 gms of spice sample put into blender Jar add 70 ml of methanol followed by 30 ml of 1% Sodium Bicarbonate into the jar mix thoroughly, blend at high speed for 1 min filter the extract through fluted filter paper (240mm) into a 250 ml beaker, after this pipette 10 ml of the filtrate into 50ml of graduated cylinder (if the sample is Nutmeg , Oregano or Black pepper add 40 ml of 20% tween 20 solution to the graduated cylinder, if the sample is not one of the product add 40ml of 1X buffer solution and mix thoroughly now transfer the content through microfiber filter paper, this will be used for clean up.

Immunoaffinity column cleanup

Immunoaffinity column cleanup (IAC); for immunoaffinity column clean up attach syringe column to pump stand rinse it with HPLC grade Methanol and then with sample solution connect OTA immunoaffinity column (Ochra Test™ column was used for IAC clean up), pipette out 10 ml of sample solution allow it to absorb on the column once entire sample is passed, rinse it with 10 ml of 1X buffer and 10ml of HPLC water place the Stoppered test tube under the column tip and add 1ml of 98:2 HPLC Methanol: Acetic Acid to it, collect the Methanol eluent in test tube and add 1ml of HPLC water to column collect the water eluent in the same vertex the mixture for 1 min, this eluent mixture is used for injection.

Instrumental conditions

Ochratoxin–A was quantified by using following instrumental conditions HPLC with fluorescent detector Excitation 333 nm and Emission 443 nm, column C18 ; 4.6 X 50 mm (Part no.: 00B-4252-EO) at ambient temperature , 51% HPLC Water+48.8% HPLC grade Acetonitril + 0.2% HPLC grade Acetic Acid was used as mobile phase; flow rate: 1ml/min injection volume: 50µl and average % of recovery Value (mean) is 87%, Limit of Quantification is 5.0 µg/Kg, Supelco Ochratoxin –A standard used as a standard for Ochratoxin- A analysis, working standard 10 ppb.

RESULTS AND DISCUSSION

In the present investigation a total of 20 spice samples belonging to 5 different spices were analysed to check the Ochratoxin-A concentration by HPLC method, in this method the selectivity is achieved by OTA immune affinity column clean up. The mean recovery value of OTA is 87% were the OTA limit of Quantification (LOQ) is 5.0 µg/Kg., if the analysed OTA concentration is below 5.0 µg/Kg then it is reported as < 5.0 µg/Kg.

A total 20 samples are analyzed for Ochratoxin-A concentration belonging to 5 spices Viz. Cardamom, Coriander, Cumin, Indian Cassia and Black Pepper from four different places by random sampling out of the 20 analysed samples (Table No.-I) 19 samples viz., Cardamom (sample codes OTAS4-fig. No.I(B), OTAS9-fig. No. I(C), OTAS14-fig. No. I(D), OTAS19-fig. No. I(E), Coriander (sample codes OTAS2- fig. No.II(A), OTAS7-fig. No. II(B), OTAS12-fig. No. II(C), OTAS17-fig. No. II(D), Cumin (sample codes OTAS1- fig. No. III(A), OTAS6-fig. No. III(B), OTAS11-fig. No. III(C), OTAS16-fig. No. III(D), Indian cassia (sample codes OTAS5-fig. No. IV(A), OTAS10-fig. No. IV(B), OTAS15-fig. No. IV(C), OTAS20-fig. No. IV(D) and Black pepper (sample codes OTAS3-fig. No.V(A), OTAS8-fig. No. V(B), OTAS13-fig. No. V(C) samples showing the total Ochratoxin-A concentration < 5.0 µg/Kg but one Black pepper sample, sample code OTAS18-fig. No. V(D) shows Ochratoxin-A concentration 5.9 µg/Kg. Fig. No. I(A) shows Ochratoxin- A Standard HPLC Chromatogram.

Table No. I Occurrence Ochratoxin A in Spice samples from Dharwad

Sl. No.	Sample Name	Sample Code	Ochratoxin-A Concentration
1	Cardamom	OTAS4	< 5.0 µg/Kg
2	Cardamom	OTAS9	< 5.0 µg/Kg
3	Cardamom	OTAS14	< 5.0 µg/Kg
4	Cardamom	OTAS19	< 5.0 µg/Kg
5	Coriander	OTAS2	< 5.0 µg/Kg
6	Coriander	OTAS7	< 5.0 µg/Kg
7	Coriander	OTAS12	< 5.0 µg/Kg
8	Coriander	OTAS17	< 5.0 µg/Kg
9	Cumin	OTAS1	< 5.0 µg/Kg
10	Cumin	OTAS6	< 5.0 µg/Kg
11	Cumin	OTAS11	< 5.0 µg/Kg
12	Cumin	OTAS16	< 5.0 µg/Kg
13	Indian Cassia	OTAS5	< 5.0 µg/Kg
14	Indian Cassia	OTAS10	< 5.0 µg/Kg
15	Indian Cassia	OTAS15	< 5.0 µg/Kg
16	Indian Cassia	OTAS20	< 5.0 µg/Kg
17	Black Pepper	OTAS3	< 5.0 µg/Kg
18	Black Pepper	OTAS8	< 5.0 µg/Kg
19	Black Pepper	OTAS13	< 5.0 µg/Kg
20	Black Pepper	OTAS18	5.9 µg/Kg

Fig. No. I (A)

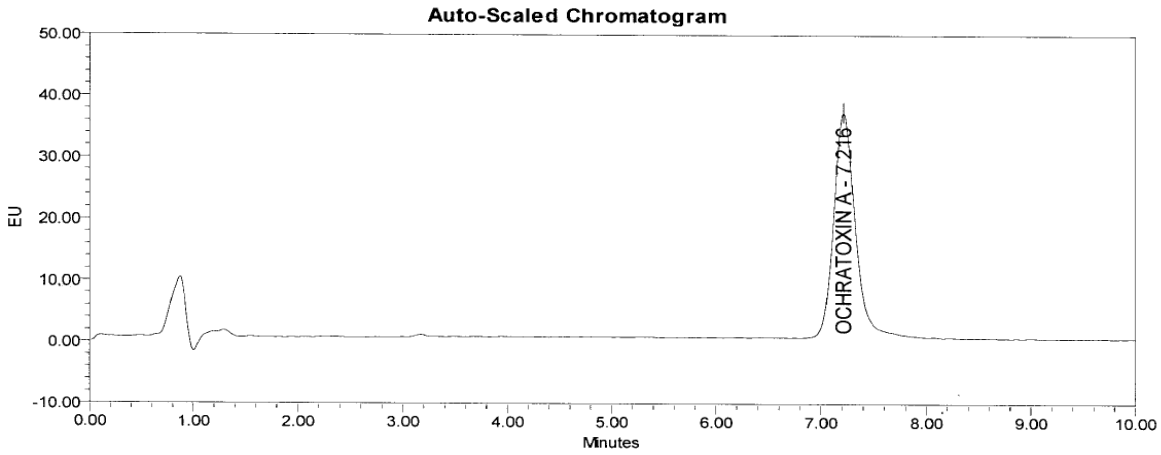


Fig. No. I (B)

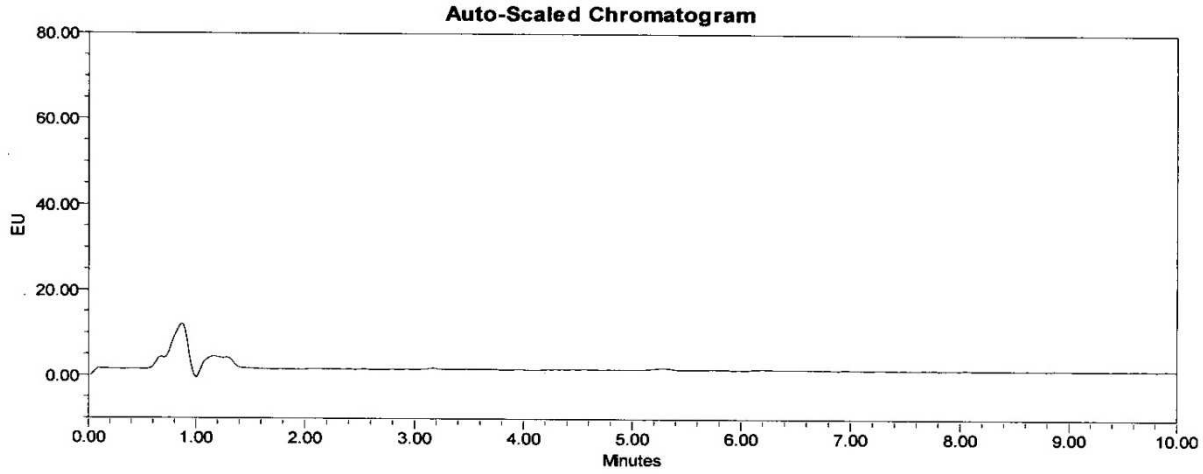


Fig. No. I (C)

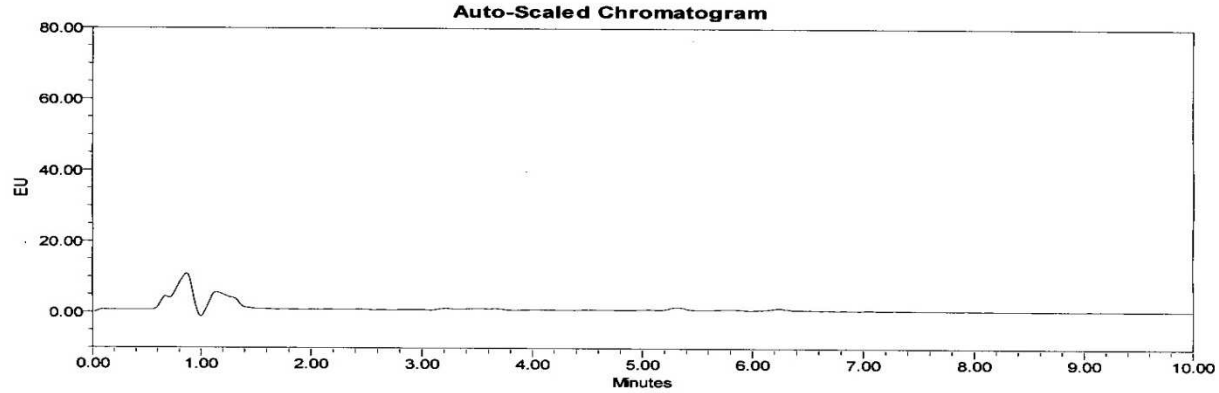


Fig. No. I (D)

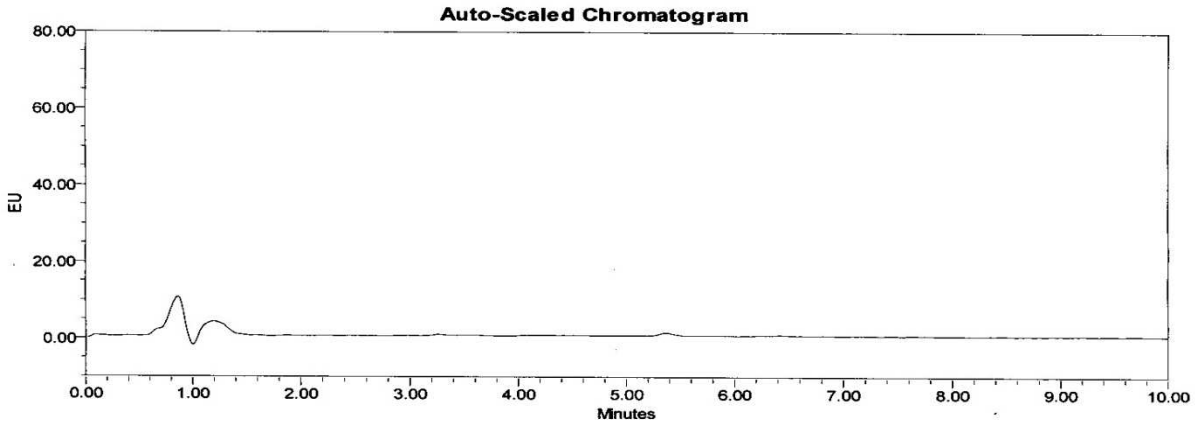


Fig. No. I (E)

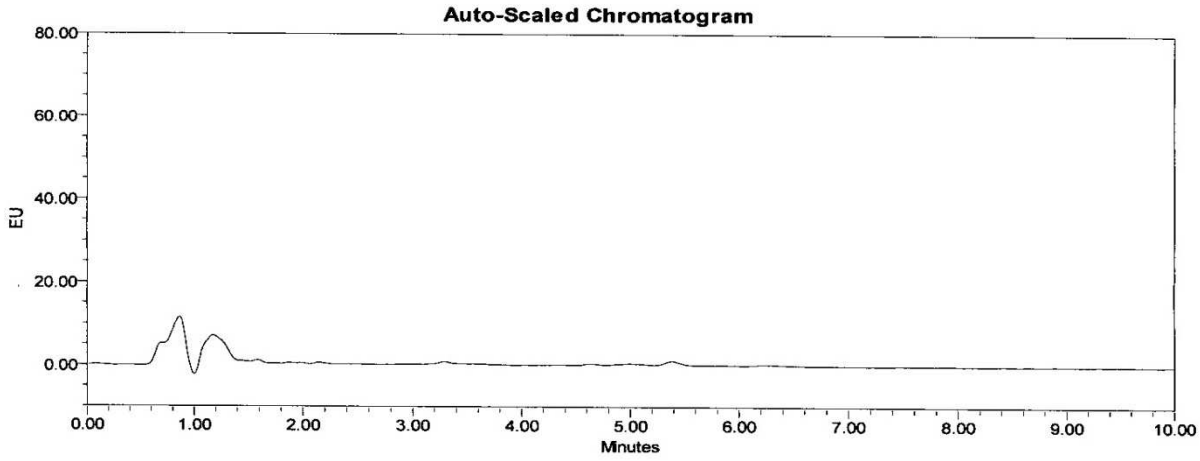


Fig. I. HPLC-FD chromatograms

(A) OTA standard Chromatogram (100 µg/Kg). (B) Cardamom sample code - OTAS4 (<5.0 µg/Kg) (C) Cardamom sample code - OTAS9 (<5.0 µg/Kg) (D) Cardamom sample code - OTAS14 (<5.0 µg/Kg) (E) Cardamom sample code - OTAS19 (<5.0 µg/Kg)

Fig. No. II (A)

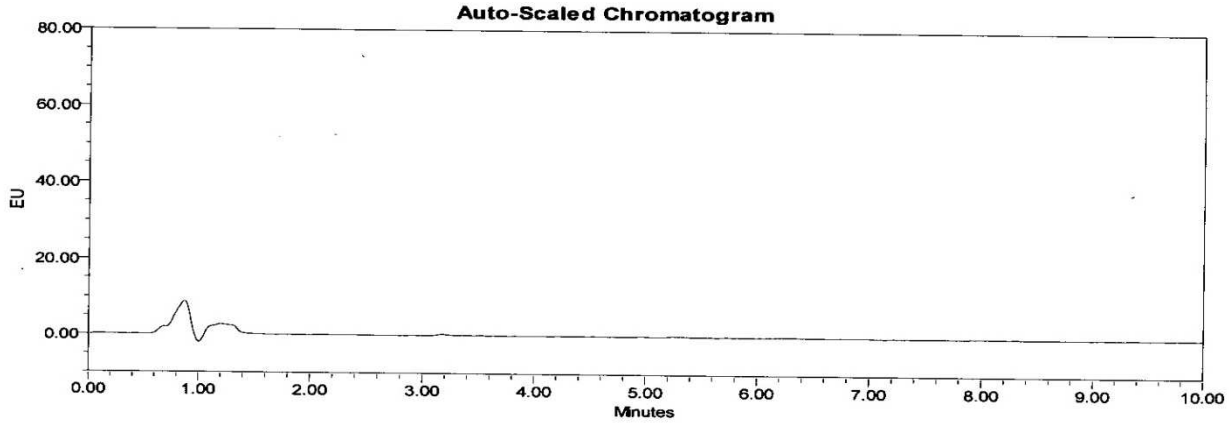


Fig. No. II (B)

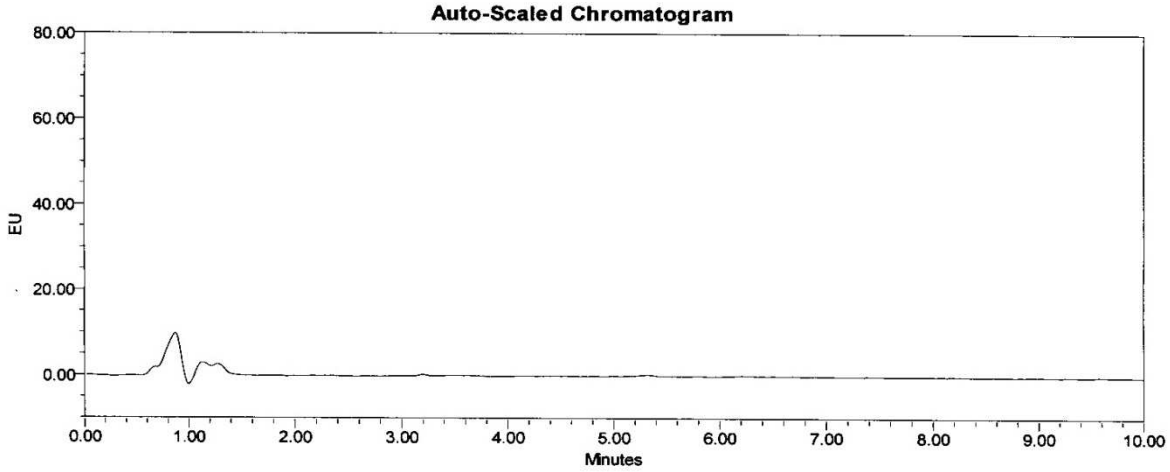
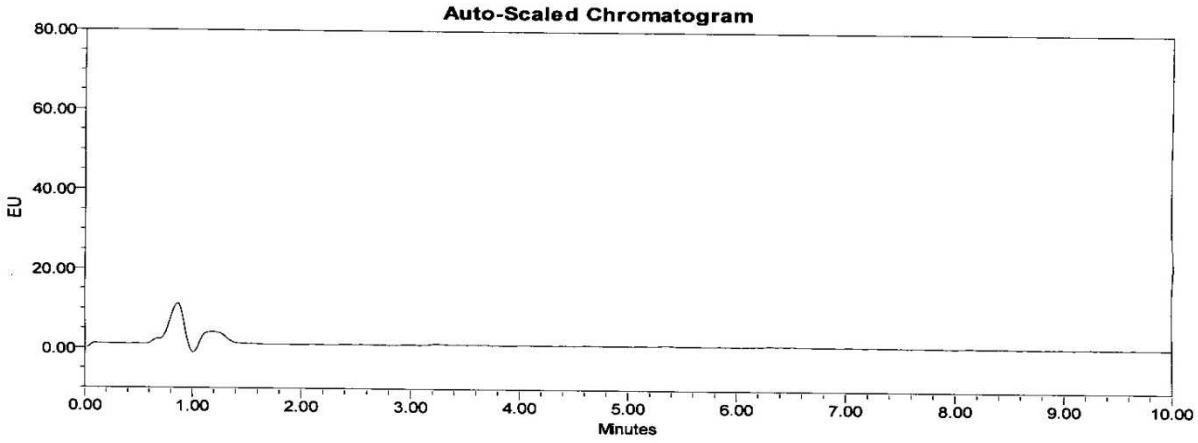


Fig. No. II (C)



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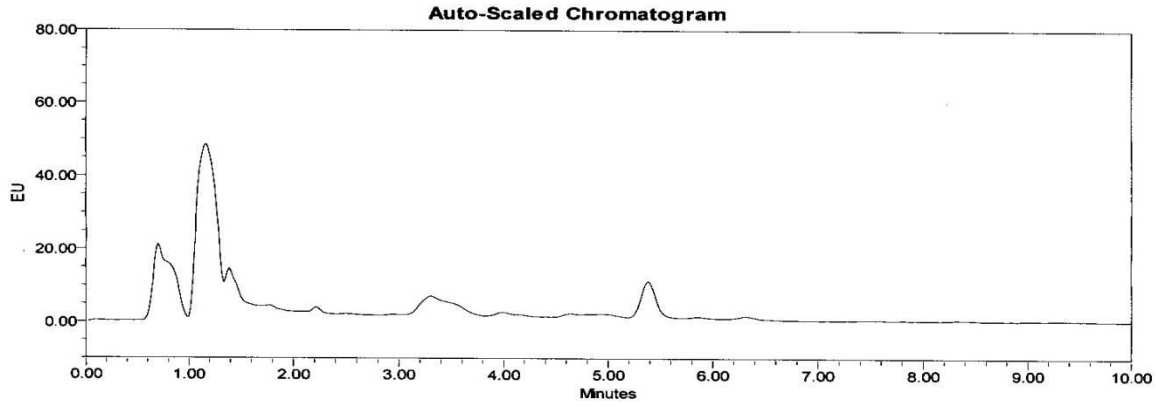


Fig. II. HPLC-FD chromatograms
(A) Coriander sample code- OTAS2 (<5.0 µg/Kg) (B) Coriander sample code- OTAS7 (<5.0 µg/Kg) (C) Coriander sample code- OTAS12 (<5.0 µg/Kg) (D) Coriander sample code- OTAS17 (<5.0 µg/Kg)

Fig. No. III (A)

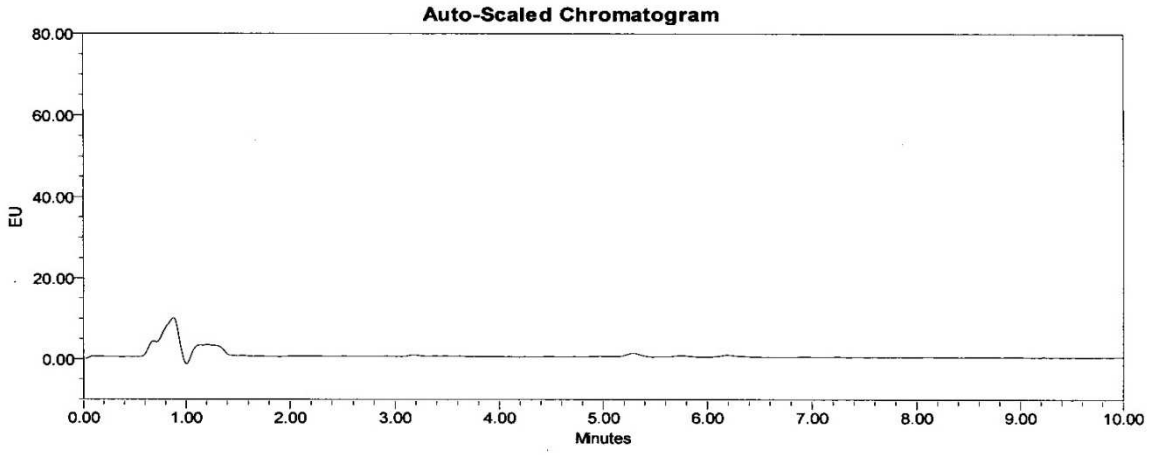


Fig. No. III (B)

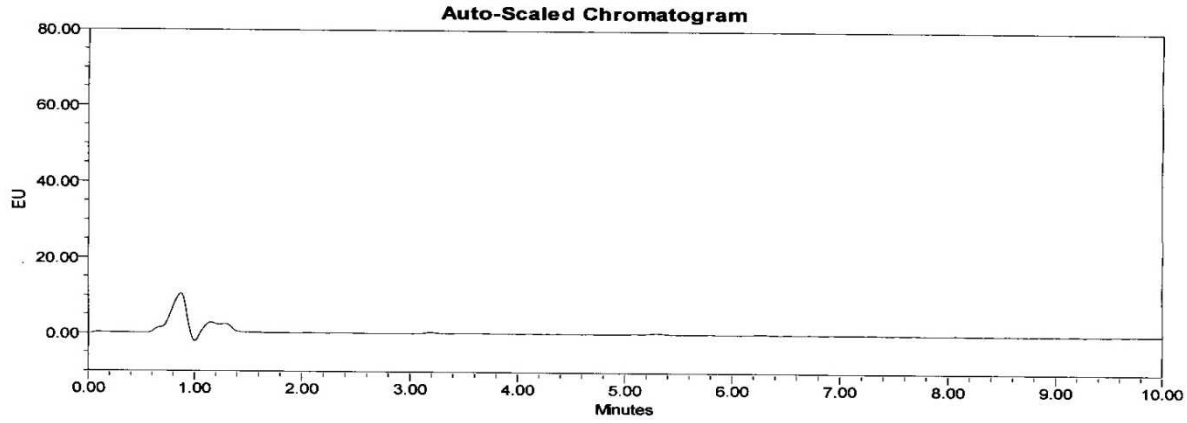


Fig. No. III (C)

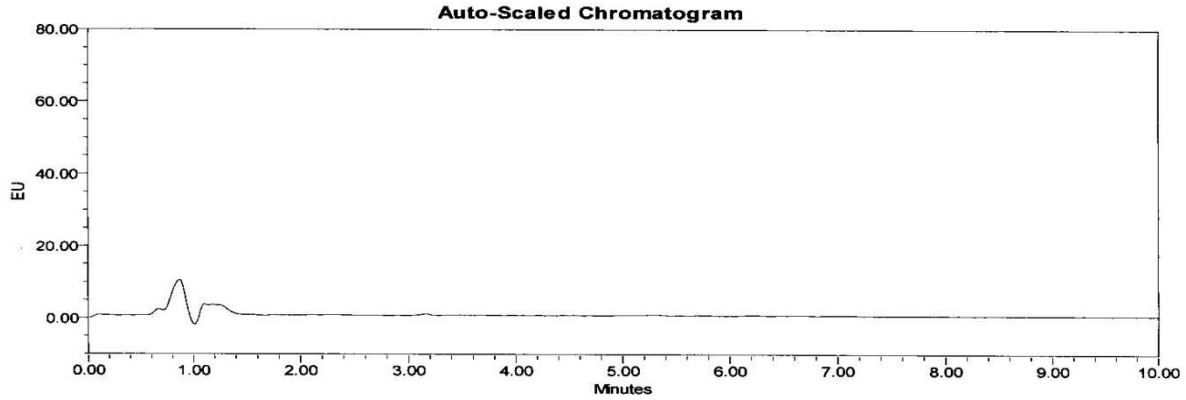


Fig. No. III (D)

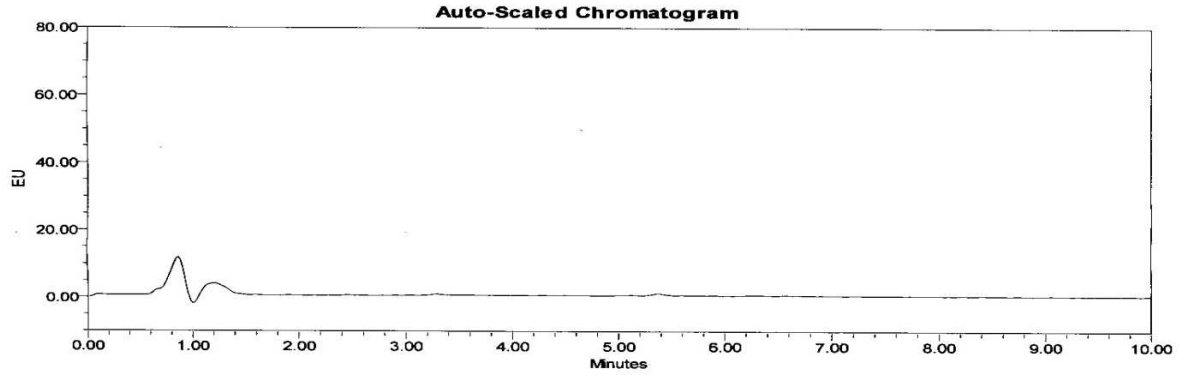


Fig. III. HPLC-FD chromatograms
(A) Cumin sample code- OTAS1 (<5.0 µg/Kg) (B) Cumin sample code- OTAS6 (<5.0 µg/Kg) (C) Cumin sample code- OTAS11 (<5.0 µg/Kg) (D) Cumin sample code- OTAS16 (<5.0 µg/Kg)

Fig. No. IV (A)

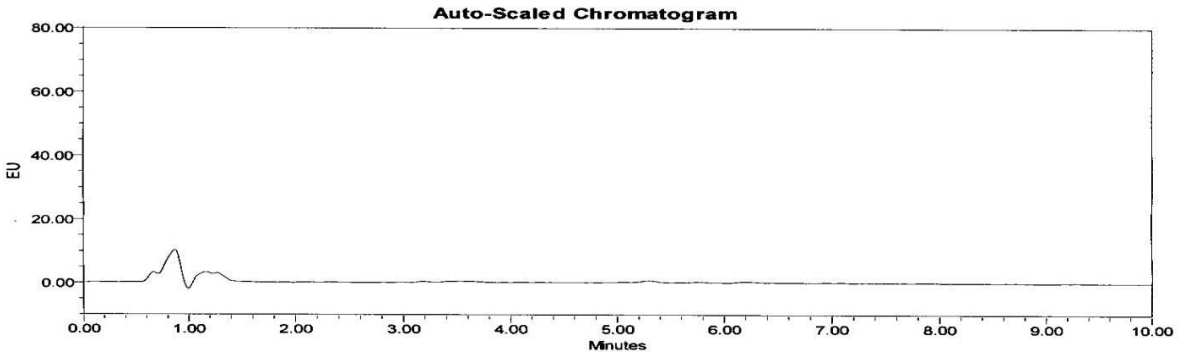


Fig. No. IV (B)

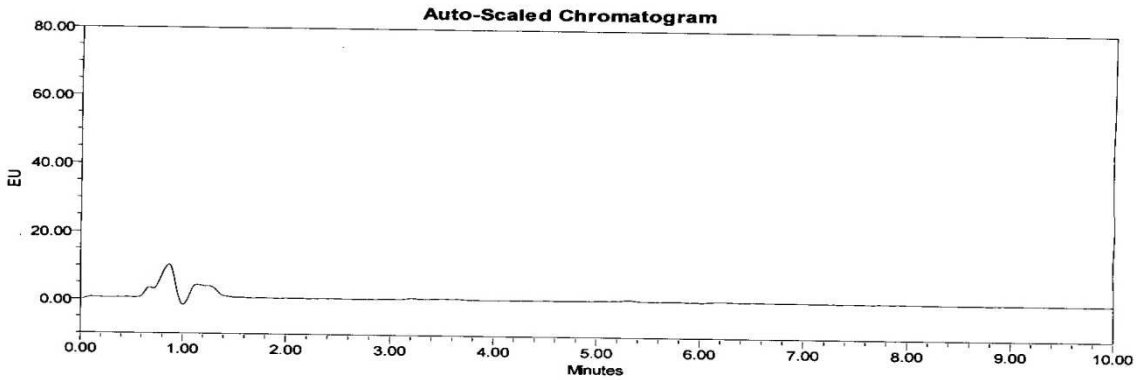
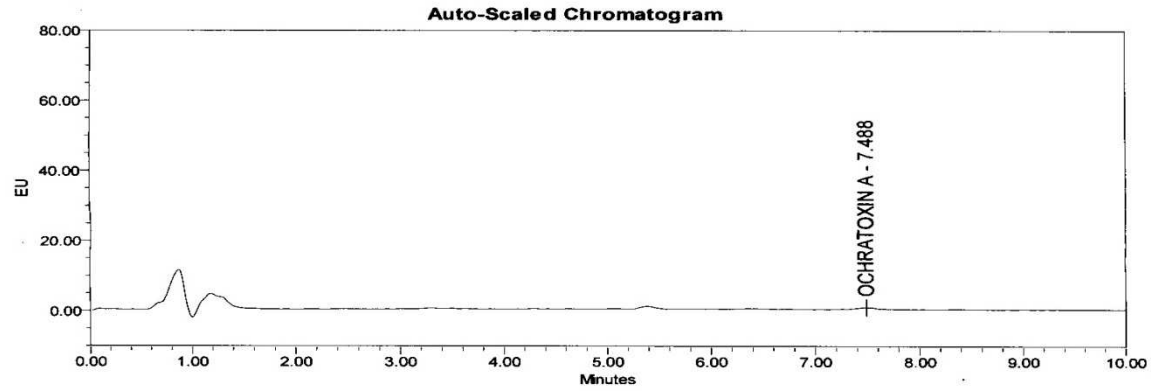


Fig. No. IV (C)



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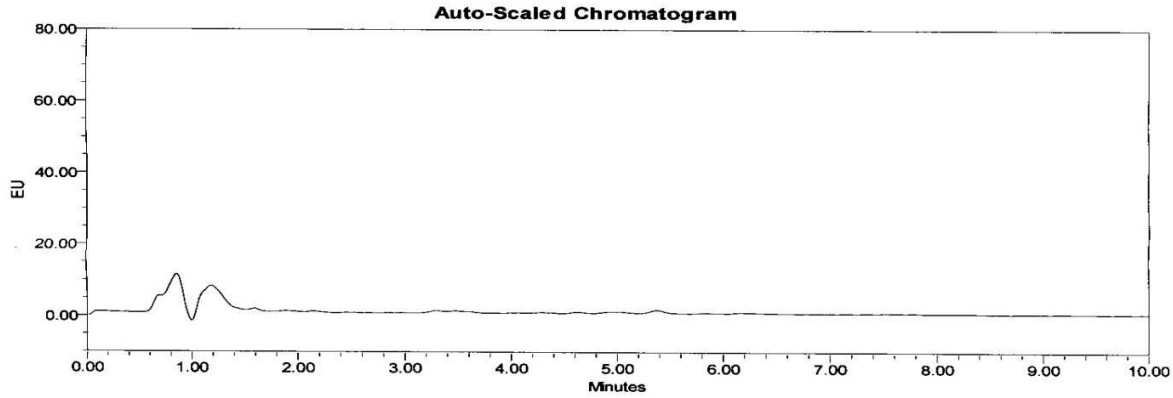

Fig. IV. HPLC-FD chromatograms

(A) Indian cassia sample code- OTAS5 (<5.0 µg/Kg) (B) Indian cassia sample code- OTAS10 (<5.0 µg/Kg) (C) Indian cassia sample code- OTAS15 (<5.0 µg/Kg) (D) Indian cassia sample code- OTAS20 (<5.0 µg/Kg)

Fig. No. V (A)

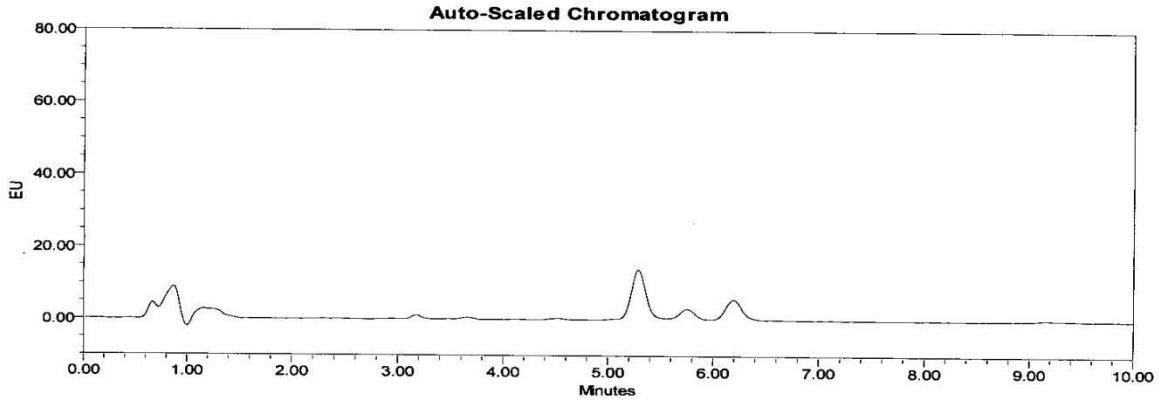

Fig. No. V (B)

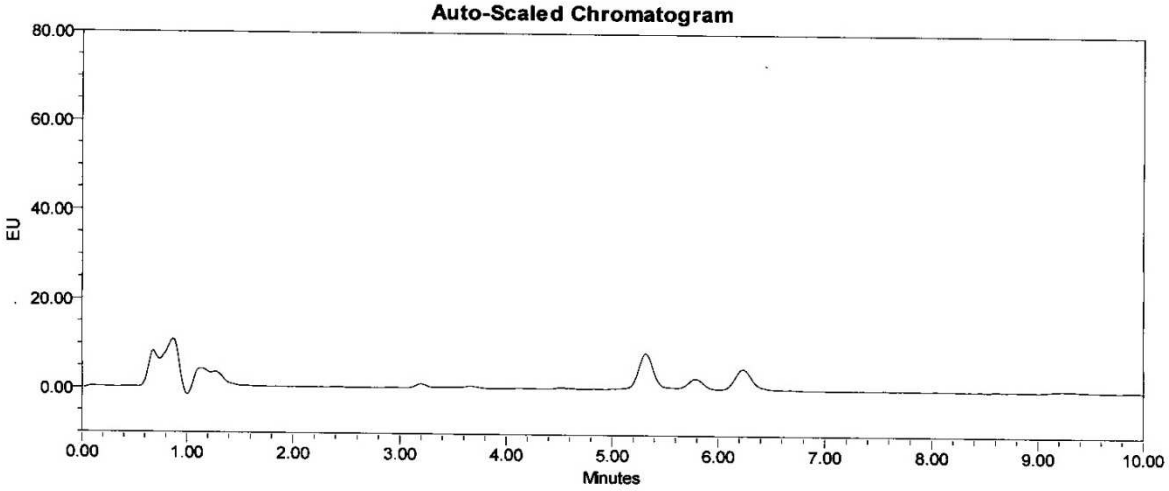

Fig. No. V (C)

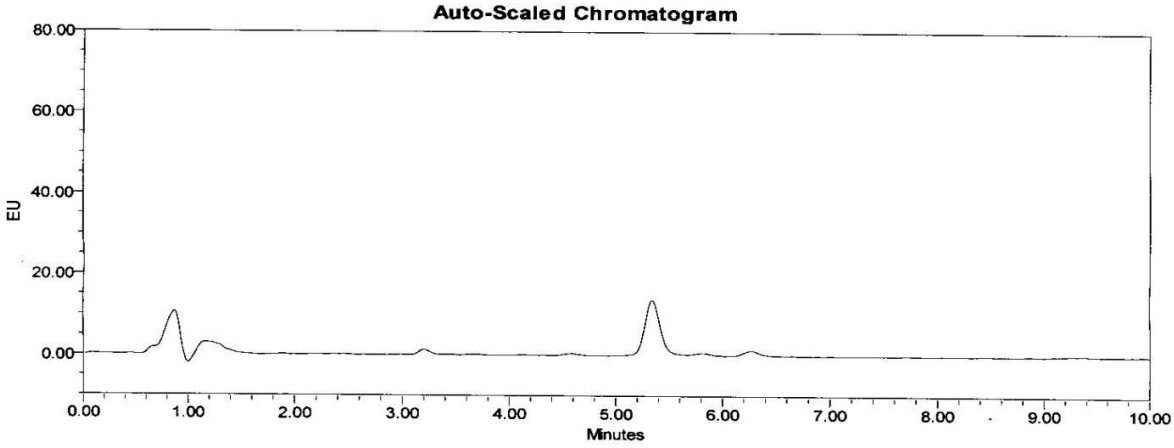

Fig. No. V (D)

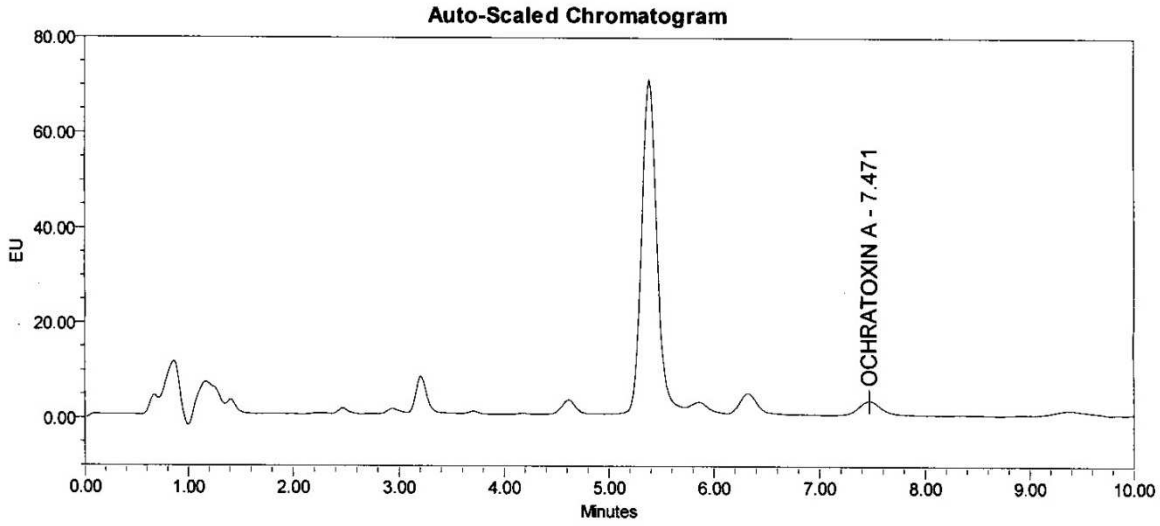

Fig. V. HPLC-FD chromatograms

(A) Black pepper sample code- OTAS3 (<5.0 µg/Kg) (B) Black pepper sample code- OTAS8 (<5.0 µg/Kg) (C) Black pepper sample code- OTAS13 (<5.0 µg/Kg) (D) Black pepper sample code- OTAS18 (5.9 µg/Kg)

Ochratoxin A contamination of various food stuffs has been reported from the various parts of the world, **[8]** reported natural occurrence of Aflatoxin and Ochratoxin A in commercial Chilli and Chilli sauce samples, Co-occurrence of Alatoxin and ochratoxin A in spices from turkey market **[9]** OTA in Black and white pepper products **[10],** occurrence of Ochratoxin A in Korean red paprika and prevention strategies factors were explained by **[11]**, **[12]** analysed OTA levels in spices and dried nuts in Tunisia market, survey of Ochratoxin A in rice from Iran **[13],** Ochratoxin- A levels in food stuffs **[14],** Natural occurrence of ochratoxin- A in some marketed Nigerian foods **[15]** Determination of OTA in food by HPLC – **[6],** Study of Ochratoxin: A complexation in coffee **[16].**

All the 20 analyzed samples showed the ochratoxin A concentration within EU consumption limit, European regulation **[17]** for ochratoxin A consumption limit for spices is 15.0 µg/Kg.

CONCLUSION

In the present investigation ochratoxin A concentration of 20 spice samples belongs tom 5 different spices viz., *Elettaria cardamomum*, Maton. (Cardamom), *Coriandrum sativum*, L. (Coriander), *Cuminum cyminum*, L. (Cumin), *Cinnamomum tamala*, T. Nees and C.H. Eberm (Indian cassia) and *Piper nigrum*, L. (Black pepper) of unknown variety were randomly collected from four different places from Dharwad. All the 20 analysed samples shows the ochratoxin A content within the EU limit only i.e. 15.0 µg/Kg for spices, Among the 20 analysed samples 19 samples viz., Cardamom (sample codes OTAS4-fig. No. I(B), OTAS9-fig. No. I(C), OTAS14-fig. No. I(D), OTAS19-fig. No. I(E), Coriander (sample codes OTAS2-fig. No. II(A), OTAS7-fig. No. II(B), OTAS12-fig. No. II(C), OTAS17-fig. No. II(D), Cumin (sample codes OTAS1-fig. No. III(A), OTAS6-fig. No. III(B), OTAS11-fig. No. III(C), OTAS16-fig. No. III(D) ,Indian cassia (sample codes OTAS5-fig. No. IV(A), OTAS10-fig. No. IV(B), OTAS15-fig. No. IV(C), OTAS20-fig. No. IV(D) and Black pepper (sample codes OTAS3-fig. No. V(A), OTAS8-fig. No. V(B), OTAS13-fig. No. V(C) samples showing the total Ochratoxin-A concentration <5.0 µg/Kg but 1 Black pepper sample, sample code OTAS18-fig. No. V(D) shows Ochratoxin- A concentration 5.9 µg/Kg and Ochratoxin- A Standard HPLC Chromatogram Fig. No. I(A) so all the 20 analysed samples from Dharwad shows the Ochratoxin-A content within the EU consumption limit (for spices is 15.0 µg/Kg).

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