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Traditional Chinese medicine ~~-~~— Determination of ~~Ochratoxin~~ochratoxin A in natural ~~pro duct~~products by LC-FLD

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Foreword

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This document was prepared by Technical Committee ~~*[for Project Committee]*~~ ISO/TC 249, *Traditional Chinese medicine*.

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Introduction

Ochratoxins are naturally occurring mycotoxins produced by certain fungi, which can be found in a variety of agriculture products, contaminated foods and natural medicines, including natural products, decoction pieces and manufactured products. Ochratoxins are a class of compounds produced by a variety of *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium sp*. According to their discovery sequence, they are called ochratoxin A (OTA), ochratoxin B (OTB) and ochratoxin C (OTC). Among these, OTA is considered the most toxic. It has been well established that OTA is highly toxic and carcinogenic. The toxicity of OTA to animals and humans mainly includes kidney toxicity, liver toxicity, teratogenesis, carcinogenesis, mutation and immunosuppression. There are frequent reports of detection of toxic OTA in natural products. Therefore, there is a need to standardize the test method of OTA in natural products, which will bring benefits to the enterprises and companies in processing, management and trade of natural products. There are two main methods to detect OTA in natural products: the liquid chromatography tandem mass spectrometry (LC-MS/MS) method and the liquid chromatography coupled with fluorescence detector (LC-FLD) method. LC-FLD is ~~preferentially chosen~~preferred due to its high sensitivity, high accuracy and reasonable operating cost.

As national implementation can differ, examples of national, regional and organizational analytical methods and values are given in Annex A and Annex C.

Traditional Chinese medicine ~~.~~— Determination of ~~Ochratoxin~~ochratoxin A in natural ~~product~~products by ~~LC-FLD~~liquid chromatography coupled with fluorescence detector

1 Scope

This document specifies the determination of ~~Ochratoxin~~ochratoxin A (OTA) in natural products ~~using LC-FLD~~by the liquid chromatography coupled with fluorescence detector (LC-FLD) method.

It is applicable to the analysis of OTA in raw materials and manufactured products, including decoction pieces derived from plants and animals. It is suitable for ~~the~~ samples during the processes of harvesting, transportation~~,~~ and storage, as well as domestic and foreign ~~trades~~trade for quality classification.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

For the purposes of this document, the following terms and definitions apply.

ISO and IEC maintain ~~terminological~~terminology databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at https://www.electropedia.org/

3.1 ~~Ochratoxin~~
~~Mycotoxins~~ochratoxin
mycotoxin produced mainly by *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium sp*~~.~~

Note 1 to entry: At least ~~7~~seven different types of ochratoxins are produced ~~in nature, and OTA~~naturally. Ochratoxin A is known to be highly toxic and carcinogenic.

Note 2 to entry: ~~OTA~~Ochratoxin A shall be tested and limited.

4 ~~Symbols and abbreviated~~Abbreviated terms

~~OTA Ochratoxin A~~

~~HPLC High Performance Liquid Chromatography~~

~~LC-FLD Liquid chromatography coupled with fluorescence detector~~

~~5~~

HPLC	high-performance liquid chromatography
LC-FLD	liquid chromatography coupled with fluorescence detector
MRL	maximum residue limit
OTA	ochratoxin A

5 Reagents

The purity of the reagents used shall be checked by running a blank determination. The chromatogram obtained from the solvents shall have a baseline without noticeable peaks that would interfere with targeted OTA.

5.1 Water, of appropriate purity (the resistivity of water shall be at least 18~~.~~,2 MΩ).

5.2 Methanol, CH_3OH, of HPLC grade.

5.3 Acetonitrile, CH_3CN, of HPLC grade.

5.4 Sodium hydrogen carbonate, $NaHCO_3$, of AR grade.

~~Note 1 to entry:~~ NOTE AR is analytical grade.

6 ~~6~~ Apparatus

~~6.1 LC-FLD~~

The ~~LC-FLD~~usual laboratory apparatus ~~consists~~and, in particular, the following shall be used.

6.1 LC-FLD, consisting of a solvent pump system, a sample injector, a chromatographic column (a column temperature controller may be used), a detector and a data acquisition system (or an integrator~~,~~ or a chart recorder). The mobile phase is supplied from one or several reservoirs and flows through the column and detector at a constant flow rate. The detector shall be a fluorescence detector.

6.2 Chromatographic column

~~A~~, of stainless steel ~~column~~ sealed with octadecylsilyl silica gel, for the chromatography test ~~shall be used~~.

6.3 Glassware

~~All glassware~~, which shall be thoroughly cleaned before use. The glassware used for OTA analysis shall be placed in a specific container filled with 0~~.~~,5 to 1~~.~~,0 % sodium hypochlorite solution for more than 2 h and then ~~shall be~~ washed with an adequate amount of fresh running water. Finally, all glassware shall be rinsed with distilled water and dried before use.

6.4 Electronic balance

~~The electronic balance shall be~~, accurate to a minimum of 0~~.~~,01 mg.

6.5 Homogenizer

~~The homogenizer shall have~~ , with a rotation speed of up to 15 ~~,~~000 ~~r/min.~~ rpm.

6.6 Centrifuge

~~The centrifuge shall have~~ , with a rotation speed of up to 5 ~~,~~000 ~~r/min~~ rpm.

6.7 ~~Volumetric flask~~

Volumetric flasks, with capacity of 2~~.~~,0 ml and 50~~.~~,0 ml ~~shall be used.~~ .

7 ~~7~~ Sampling preparation

~~1)~~ a) All natural products shall be crushed into powders and screened through a 24-mesh sieve.

~~2) Mixture~~b) A mixture of 25~~.~~,0 g powders and 4~~.~~,0 g sodium chloride shall be added ~~into~~to a 85~~.~~,0 ml mixed solution of methanol and water ~~at~~ in a volume ratio of 80:20~~, v/v~~.

~~3) All the~~c) The mixture shall be homogenized at a speed ~~of~~ higher than 11 ~~,~~000 rpm for 2 min~~,~~ and centrifuged at 4 ~~,~~000 rpm for 10 min.

~~4)~~ d) 10~~.~~,0 ml of supernatant shall be moved to a 50~~.~~,0 ml volumetric flask ~~and~~, diluted with water~~,~~ ~~and~~ then centrifuged at 4 ~~,~~000 rpm for 10 min.

~~5)~~ e) About 10~~.~~,0 ml of the filtrate shall be passed through the immunoaffinity column at a flow rate of 3 ml/min. The column shall be washed with 20~~.~~,0 ml of water~~,~~ and the eluent shall be abandoned until the air has passed through the column to extrude the water.

~~6)~~ f) The column shall be eluted with methanol~~,~~ and the eluent ~~shall be~~ collected and diluted with methanol in a 2~~.~~,0 ~~mL~~ ml volumetric flask.

~~7)~~ g) 2~~.~~,0 ml of solution shall be filtered through a 0~~.~~,22 μm filter paper~~,~~ and the filtrate shall be used in the following analysis.

8 ~~8~~ Test method

8.1 ~~8.1~~ Stock solution and working solution

Stock solution shall be prepared by a solution of OTA standards. A series of working solutions shall be prepared by diluting the stock solution to 0~~.~~,1 ~~–~~ ng/ml to 100~~.~~,0 ng/ml with mobile phase of methanol.

8.2 ~~8.2~~ LC-FLD conditions

8.2.1 General

The LC-FLD method ~~was~~is used ~~to~~for the determination of OTA based on the visible fluorescence stimulated by light of appropriate wavelength. The LC-FLD method ~~has received wide application~~is widely used in many countries, including ~~Europe~~Germany, China~~,~~ and America ~~and Germany~~. In the published documents, the method has been applied in a variety of matrices, including feed, foodstuff,

~~even in~~ ginseng and turmeric. Hence, the LC-FLD can be recommended as ~~an international standard~~a method for the determination of OTA in natural products.

~~8.2.1~~8.2.2 ~~8.2.1~~ LC-FLD conditions and system suitability

a) A stainless~~-~~steel column sealed with octadecylsilyl silica gel for chromatography measurement shall be used.

b) The mobile phase of acetonitrile-glacial acetic acid-water shall be used for isocratic elution.

c) The flow rate of the mobile phase is set ~~at~~to 1~~.~~,0 ~~mL~~ ml/min.

d) The excitation wavelength, λ_{ex}, and the emission ~~wavelengths~~wavelength, λ_{em}, of the fluorescence detector shall be set ~~at~~ λ_{ex}~~=~~to 333 nm and ~~λ_{em}~~=477 nm, respectively.

e) The resolution of two adjacent chromatographic peaks should be higher than 1~~.~~,5.

~~Note 1 to entry: λ_{ex} is excitation wavelength (nm) of the fluorescence detector, and λ_{em} is emission wavelength (nm) of the fluorescence detector.~~

~~8.2.2~~8.2.3 ~~8.2.2~~ Quantification of OTA in the test sample using calibration curves

25 ~~μL~~ μl of each working solution of standard solutions shall be injected into the LC-FLD system to record the peak area of OTA~~,~~ and the chromatogram of OTA ~~could~~can refer to Annex B. The calibration curves of OTA shall be established by plotting peak area versus the serially diluted concentration of OTA. Afterwards, the test sample solution ~~also~~ shall also be injected into the LC-FLD system to record the peak area of OTA. Then~~,~~ the contents of OTA in test samples shall be calculated using ~~above mentioned~~these calibration curves.

8.3 ~~8.3~~ Application of test method

The described method has been shown to be suitable for liquorice extract and liquorice root (~~See~~see Annex C). This method can also be used for the other kinds of natural ~~product,~~products but it shall be demonstrated by method validation.

9 ~~9~~ Sampling and preservation

9.1 ~~9.1~~ Sampling

For each batch, the following quantities of samples shall be used: no less than 100 g of general medicinal materials and decoction pieces; no less than 25 g of powdered medicinal materials and decoction pieces; 5 g of precious medicinal materials and decoction pieces.

Natural product samples received ~~into~~by the laboratory shall be labelled with complete information, such as the collected source, date and time, the correct species of material~~,~~ and the name of the appraiser. The testing samples shall ~~be included~~include whole medicinal materials and decoction pieces derived from plants or animals.

On receipt, a sample shall immediately be assigned a unique identification code, which shall be accompanied through all stages of the analysis to the reporting of the results. Records of samples shall be kept ~~by~~according to specified person and place.

9.2 ~~9.2~~ Sample storage

Before testing, the sample shall be dried and powdered. Samples shall be prepared immediately and ~~recommended to~~should be stored in a dark place at 4 ~~°C in refrigerator~~ °C.

If samples cannot be analysed immediately, they shall be stored below 4 ~~°C~~ °C away from sunlight. The mass of the flask shall be recorded before and after each measurement of the solution.

~~Annex A~~