
**Traditional Chinese medicine —
Determination of ochratoxin A
in natural products by liquid
chromatography coupled with
fluorescence detector**

*Médecine traditionnelle chinoise — Détermination de l'ochratoxine A
dans les produits naturels par chromatographie liquide couplée à un
détecteur de fluorescence*

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Published in Switzerland

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular, the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 249, *Traditional Chinese medicine*.

Any feedback or questions on this document should be directed to the user's national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

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 consumers, 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 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](#).

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Traditional Chinese medicine — Determination of ochratoxin A in natural products by liquid chromatography coupled with fluorescence detector

1 Scope

This document specifies the determination of ochratoxin A (OTA) in natural products 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 samples during the processes of harvesting, transportation and storage, as well as domestic and foreign 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 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

mycotoxin produced mainly by *Aspergillus ochraceus*, *Aspergillus niger* and *Penicillium sp*

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

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

4 Abbreviated terms

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, CH₃OH, of HPLC grade.

5.3 Acetonitrile, CH₃CN, of HPLC grade.

5.4 Sodium hydrogen carbonate, NaHCO₃, of AR grade.

NOTE AR is analytical grade.

6 Apparatus

The usual laboratory apparatus 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, of stainless steel sealed with octadecylsilyl silica gel, for the chromatography test.

6.3 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 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, accurate to a minimum of 0,01 mg.

6.5 Homogenizer, with a rotation speed of up to 15 000 rpm.

6.6 Centrifuge, with a rotation speed of up to 5 000 rpm.

6.7 Volumetric flasks, with capacity of 2,0 ml and 50,0 ml.

7 Sampling preparation

- a) All natural products shall be crushed into powders and screened through a 24-mesh sieve.
- b) A mixture of 25,0 g powders and 4,0 g sodium chloride shall be added to a 85,0 ml mixed solution of methanol and water in a volume ratio of 80:20.
- c) The mixture shall be homogenized at a speed higher than 11 000 rpm for 2 min and centrifuged at 4 000 rpm for 10 min.
- d) 10,0 ml of supernatant shall be moved to a 50,0 ml volumetric flask, diluted with water then centrifuged at 4 000 rpm for 10 min.
- 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.
- f) The column shall be eluted with methanol and the eluent collected and diluted with methanol in a 2,0 ml volumetric flask.

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

8 Test method

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 LC-FLD conditions

8.2.1 General

The LC-FLD method is used for the determination of OTA based on the visible fluorescence stimulated by light of appropriate wavelength. The LC-FLD method is widely used in many countries, including Germany, China and America. In the published documents, the method has been applied in a variety of matrices, including feed, foodstuff, ginseng and turmeric. Hence, the LC-FLD can be recommended as a method for the determination of OTA in natural products.

8.2.2 LC-FLD conditions and system suitability

- A stainless-steel column sealed with octadecylsilyl silica gel for chromatography measurement shall be used.
- The mobile phase of acetonitrile-glacial acetic acid-water shall be used for isocratic elution.
- The flow rate of the mobile phase is set to 1,0 ml/min.
- The excitation wavelength, λ_{ex} , and the emission wavelength, λ_{em} , of the fluorescence detector shall be set to 333 nm and 477 nm, respectively.
- The resolution of two adjacent chromatographic peaks should be higher than 1,5.

8.2.3 Quantification of OTA in the test sample using calibration curves

25 μ l 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 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 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 these calibration curves.

8.3 Application of test method

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

9 Sampling and preservation

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 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 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 according to specified person and place.

9.2 Sample storage

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

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

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Annex A

(informative)

Referenced analytical methods for determining OTA in different countries and regions

There are various methods for the determination of OTA in natural products. LC-FLD is the most widely used method and the LC-MS/MS is the technique of choice for the simultaneous determination of various mycotoxins. Referenced analytical methods of determining OTA in different countries and regions are detailed in [Table A.1](#).

Table A.1 — National, regional and organizational analytical methods and application scope of determination of OTA in natural products

Country or organization	Regulation	Edition	Application scope	Method
China	Chinese Pharmacopoeia	2020	Traditional Chinese medicines	a) LC-FLD with immunoaffinity column clean-up b) LC-MS/MS with immunoaffinity column clean-up
	National standard (GB 5009.96)	2016	Cereals, beans, pepper	LC-FLD with immunoaffinity column clean-up
Association of Official Analytical Chemists (AOAC)	AOAC official method	2009	Ginseng, turmeric	LC-FLD with immunoaffinity column clean-up
Europe	European Pharmacopoeia	9.0	Herbal drugs, including liquorice extract and root	HPLC-FLD with immunoaffinity column clean-up
Germany	EN	15829–2010	Currants, raisins, sultanas, mixed dried fruit and dried figs	HPLC-FLD with immunoaffinity column clean-up
United Kingdom	British Pharmacopoeia	2017	Herbal drugs, including liquorice extract and liquorice root	LC-FLD with immunoaffinity column clean-up

Annex B (informative)

Referenced chromatogram for determination of OTA in natural products using HPLC-FLD

Figure B.1 shows a referenced chromatogram of OTA analysed by HPLC-FLD, in which one main peak is detected.

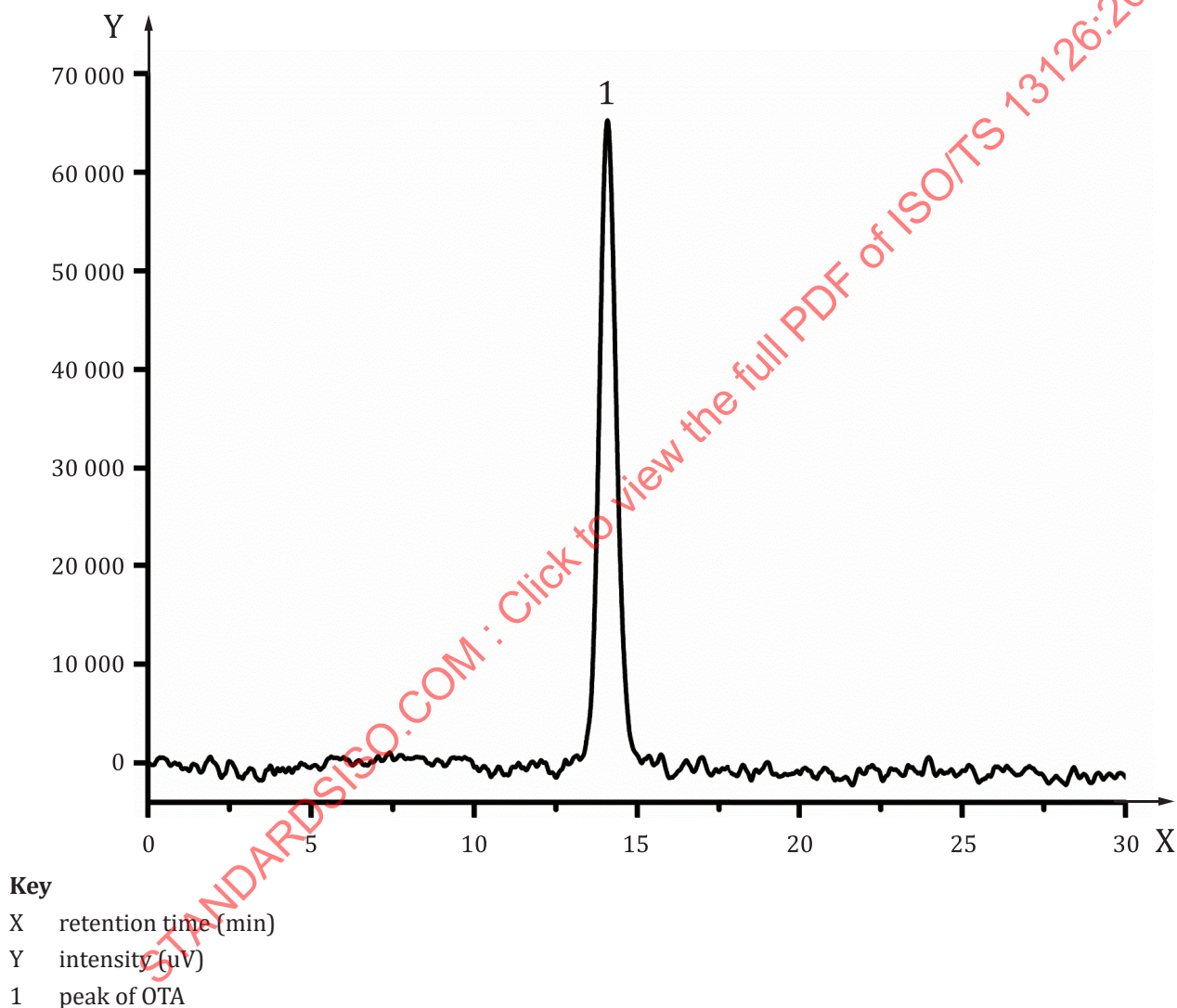


Figure B.1 — HPLC chromatogram of OTA in natural products