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Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography

Médecine traditionnelle chinoise — Détermination des résidus de pesticides dans les produits naturels par chromatographie en phase gazeuse

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Foreword

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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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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

A pesticide is any substance or mixture of substances intended to prevent, destroy or control any pest, unwanted species of plants or animals causing harm during, or otherwise interfering with, the production, processing, storage, transport or marketing of uncontaminated products. At present, there is no uniformly accepted international standard which defines maximum limits for pesticides in natural products used in traditional Chinese medicine (TCM), resulting in disputes about what levels should be considered acceptable.

This document was developed in response to worldwide demand for harmonization of the determination of pesticide residues. This document is applicable to natural products used in TCM.

For reference, the method of determination of pesticide residues by gas chromatography (GC) is provided in Annex A, the maximum limits of pesticide residues in natural products used in TCM are provided in Annex B and the recommended limits of pesticide residues in dried fruit and vegetables, which is similar to TCM materials or herbal medicine from the World Health Organization (WHO) and Food and Drug Administration/Environmental Protection Agency (FDA/EPA), are given in Annex C.

Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography

1 Scope

This document specifies the method of determination of pesticide residues in natural products used in traditional Chinese medicine (TCM) by gas chromatography (GC), including Chinese materia medica (whole medicinal materials) and decoction pieces derived from plants.

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 databases for use in standardization at the following addresses:

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

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

3.1
pesticide
substance or mixture of substances intended for preventing, destroying, repelling or reducing any pest or weeds

[SOURCE: ISO 27065:2017, 3.9, modified — Note 1 to entry removed.]

3.2
pesticide residue
pesticide, pesticide derivative or pesticide adjuvant that remains in or on a natural product

Note 1 to entry: Pesticide residues are expressed in mg/kg.

3.3
acceptable daily intake
ADI
estimate of the amount of a pesticide in natural products that can be safely consumed daily over a lifetime without adverse health effects

Note 1 to entry: ADI is expressed in milligrams of the pesticide, as it appears in the natural products, per kilograms of body mass per day (mg/kg/day).

4 Sampling

To reduce the effect of sampling in the determination of pesticide residues in natural products used in TCM, ensure that the composition of the sample used is representative of the batch of natural products used in TCM being examined. The sampling procedures may be used if they can be demonstrated to produce representative batch samples.

Samples shall be accompanied with complete information (e.g. name, source, specification of the samples) to ensure traceability.

5 Determination of pesticide residues

5.1 Reagents

5.1.1 General

Use reagents of purity suitable for pesticide residue analysis. All reagents shall be of recognized chromatographic or analytical purity. Avoid possible contamination of water, solvents, sorbents inorganic salts or other reagents.

5.1.2 Check for purity of reagents

When using GC 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 peak that would interfere or overlap with the peaks from the chromatogram of the targeted pesticide residue (see ISO 4389:2000, Clause 4).

5.2 Apparatus

All equipment, especially glassware, shall be thoroughly cleaned to ensure that it is free from pesticides. All glassware shall be soaked for a minimum of 16 h in a solution of phosphate-free detergent, rinsed repeatedly with distilled water to remove the detergent residue, then washed with acetone, followed by hexane or heptane. Do not use ordinary plastics, for example PVC stoppers, in vessels for storing standard materials and solutions as they can cause contamination. Polypropylene (PP), polytetrafluoroethylene (PTFE) or nylon tubing and glass or PTFE stoppers usually present the lowest risk of contamination.

Common laboratory glassware or equipment such as beakers, round-bottomed flasks, watch glasses, pipettes, filter papers, glass wool, glass rods and glass beads are not listed in method in detail.

5.3 Qualitative and quantitative analysis of pesticide residues

Validated analytical procedures that satisfy the following criteria shall be used:

1) The chosen method, especially the purification steps, is suitable for the combination pesticide residue/substance to be examined, and not susceptible to interference from co-extractives.

2) Natural occurrence of some constituents is considered in the interpretation of results (e.g. disulfide from cruciferaceae).

3) The concentration of test and reference solutions and the setting of the apparatus are such that the responses used for quantification of the pesticide residues are within the dynamic range of the detector. Test solutions containing pesticide residues at a level outside the dynamic range may be diluted within the calibration range, provided that the concentration of the matrix in the solution is adjusted in cases where the calibration solutions must be matrix-matched.

4) Between 70 % and 120 % of each pesticide is recovered; lower recoveries may be acceptable in certain justified cases.

5) Repeatability of the method: relative standard deviation (RSD) is not greater than the values indicated in Table 1.

6) Reproducibility of the method: RSD is not greater than the values indicated in Table 1.

Table 1 — The request of the repeatability and reproducibility of the method for determination of pesticide residues

Concentration range of the pesticide mg/kg	Repeatability (RSD) %	Reproducibility (RSD) %
0,001 – 0,01	30	60
> 0,01 – 0,1	20	40
> 0,1 – 1	15	30
> 1	10	20

5.4 Test

For reference, the method of determination of pesticide residues by GC is provided in Annex A. In certain cases it is possible to improve the method performance by variations in equipment used, extraction, clean-up and chromatographic conditions. Such variations shall always be clearly documented and demonstrated to give valid results.

6 Limits

Limits for pesticides are calculated using the following formula:

$$\text{Limit (mg/kg)} = Am/100B$$

where

A is the ADI, in mg/kg of body mass;

m is body mass, in kg (60 kg);

B is the daily dose of the article, in kg.

The competent authority may grant total or partial exemption from the test when the complete history (nature and quantity of the pesticides used, date of each treatment during cultivation and after the harvest) of the treatment of the batch is known and can be checked precisely according to good agricultural and collection practice (GACP).

Maximum limits of pesticide residues in natural products used in TCM are provided in Annex B.

Annex A
(informative)

Determination of pesticide residues in natural products used in TCM by GC

A.1 Extraction

Use the following procedure for the analysis of samples of articles having a water content of less than 15 %. Samples having a higher water content may be dried, provided that the drying procedure does not significantly affect the pesticide content.

Add 100 ml of acetone to 10 g of the coarsely powdered substance (pulverized through a 50-mesh sieve) under test and allow to stand for 20 min. Add 1 ml of a solution in toluene containing 1,8 µg of carbophenothion per ml. Mix in a high-speed blender for 3 min. Filter this solution and wash the residue with two 25 ml portions of acetone. Combine the filtrate and the washings and heat in a rotary evaporator, maintaining the temperature of the bath below 40 °C, until the solvent has almost completely evaporated. To the residue add a few ml of toluene and heat again until the acetone is completely removed. Dissolve the residue in 8 ml of toluene. Pass through a membrane filter of 45 µm pore size, rinse the flask and the filter with toluene, dilute with toluene to 10,0 ml (solution A) and mix.

A.2 Purification

A.2.1 Organochlorine, organophosphorus and pyrethroid pesticide residues

A.2.1.1 General

The size-exclusion chromatograph is equipped with a 7,8 mm × 30 cm stainless steel column containing rigid, spherical styrene-divinylbenzene copolymer (5 µm in diameter) as packing material. Toluene is used as the mobile phase at a flow rate of about 1 ml per min.

A.2.1.2 Performance of the column

Inject 100 µl of a solution in toluene containing, in each ml, 0,5 mg of methyl red and 0,5 mg of oracet blue or equivalent. The column is not suitable unless the colour of the eluate changes from orange to blue at an elution volume of about 10,3 ml. If necessary, calibrate the column using a solution in toluene containing suitable concentrations of the pesticide of interest with the lowest molecular weight (e.g. dichlorvos) and the highest molecular weight (e.g. deltamethrin). Determine which fraction of the eluate contains both pesticides.

A.2.1.3 Purification of the test solution

Inject a suitable volume (100 µl to 500 µl) of solution A into the chromatograph. Collect the fraction (solution B) as determined in A.2.1.2. Organophosphorus pesticides elute between 8,8 ml and 10,9 ml. Organochlorine and pyrethroid pesticides elute between 8,5 ml and 10,3 ml.

A.2.2 Organochlorine and pyrethroid pesticide residues

Into a 5 mm × 10 cm chromatographic column, introduce a piece of fat-free cotton and 0,5 g of silica gel treated as follows. Heat 0,5 g of chromatographic silica gel in an oven at 150 °C for at least 4 h. Allow to cool and add dropwise a quantity of water corresponding to 1,5 % of the mass of silica gel used. Shake

vigorously until agglomerates have disappeared and continue shaking by mechanical means for 2 h. Condition the column with 1,5 ml of hexane.

Pre-packed columns containing about 0,50 g of a suitable silica gel may also be used, provided they have been previously validated.

Concentrate solution B almost to dryness with the aid of a stream of helium or oxygen-free nitrogen and dilute with toluene to a suitable volume (200 µl to 1 ml, according to the volume injected in the preparation of solution B). Quantitatively transfer this solution to the column and proceed with the chromatography, using 1,8 ml of toluene as the mobile phase. Collect the eluate (solution C).

A.3 Quantitative analysis of organophosphorus pesticide residues

A.3.1 Test solution

Concentrate solution B almost to dryness with the aid of a stream of helium, dilute with toluene to 100 µl and mix.

A.3.2 Standard solution

Prepare at least three solutions in toluene containing each of the pesticides of interest and carbophenothion at concentrations suitable for plotting a calibration curve.

A.3.3 Chromatographic system

a) column: a fused silica column (0,32 mm × 30 m) with a film (0,25 µm) of dimethylpolysiloxane oil;

b) detector: alkali flame ionization detector (AFID) or flame photometric detector (FPD);

c) injection port temperature: 250 °C;

d) detector temperature: 275 °C;

e) column temperature program: maintain the initial temperature at 80 °C for 1 min, then increase to 150 °C at a rate of 30 °C per min; maintain at 150 °C for 3 min, then increase to 280 °C at a rate of 4 °C per min; maintain at this temperature for 1 min.

Hydrogen is used as the carrier gas. Other gases, such as helium or nitrogen, may also be used. Use carbophenothion as the internal standard. If necessary, use a second internal standard to identify any possible interference with the peak corresponding to carbophenothion.

Inject the chosen volume of each solution, record the chromatograms and measure the peak responses. Calculate the content of each pesticide from the peak areas and the concentrations of the solution.

A.4 Quantitative analysis of organochlorine and pyrethroid pesticide residues

A.4.1 Test solution

Concentrate solution C almost to dryness with the aid of a stream of helium or oxygen-free nitrogen, dilute with toluene to 500 µl and mix.

A.4.2 Standard solution

Prepare at least three solutions in toluene containing each of the pesticides of interest and carbophenothion at concentrations suitable for plotting a calibration curve.

A.4.3 Chromatographic system

a) column: a fused silica column (0,32 mm × 30 m) with a film (0,25 µm) of dimethylpolysiloxane oil;