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Traditional Chinese medicine — *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root

Médecine traditionnelle chinoise — Racine de Bupleurum chinense, Bupleurum scorzonerifolium *et* Bupleurum falcatum

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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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

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

Bupleurum chinense, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root, namely Bupleuri Radix, are well-known traditional Chinese materia medica which have liver-protection, antipyretic and anti-inflammatory qualities. Bupleuri Radix is sold in many countries as medicinal materials or decoction pieces. Bupleuri Radix originates from cross-pollinating plants, which can easily lead to a hybrid of their provenance, in turn resulting in mixed sources of herbs. This can lead to difficulties in herb authenticity, as well as variations in herb efficacy and safety. Bupleuri Radix is included in several pharmacopeia, but with different plant origins. Thus, there is a need to standardize the quality to bring benefits to consumers and the enterprises involved in the processing, management and trade of *Bupleurum chinense, Bupleurum scorzonerifolium* and *Bupleurum falcatum* root.

As national implementation can differ, national standards bodies can modify the values given in 5.3, 5.4, 5.5 and 5.8 in their national standards. Examples of national and regional values are given in Annex G.

Traditional Chinese medicine — *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root

1 Scope

This document specifies minimum requirements and test methods for *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root.

This document applies to *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root that are sold and used as natural medicines in international trade, including Chinese materia medica (whole medicinal materials) and decoction pieces.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

ISO 18664, *Traditional Chinese Medicine — Determination of heavy metals in herbal medicines used in Traditional Chinese Medicine*

ISO 21371, *Traditional Chinese medicine — Labelling requirements of products intended for oral or topical use*

ISO 22258, *Traditional Chinese medicine — Determination of pesticide residues in natural products by gas chromatography*

ISO 22590, *Traditional Chinese medicine — Determination of sulfur dioxide in natural products by titration*

ISO 23723, *Traditional Chinese medicine — General requirements for herbal raw material and materia medica*

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
root mass
average mass of final samples of root

Note 1 to entry: Root mass is measured in grams.

3.2
root length
largest distance from the bottom of the tap root to the stem scar

Note 1 to entry: Root length is measured in centimetres.

3.3
batch
samples collected from the same particular place at the same time

3.4
sample
representative material taken from a product, or part of a product

[SOURCE: ISO 11596:2021, 3.1.4, modified — Definition revised and note to entry removed.]

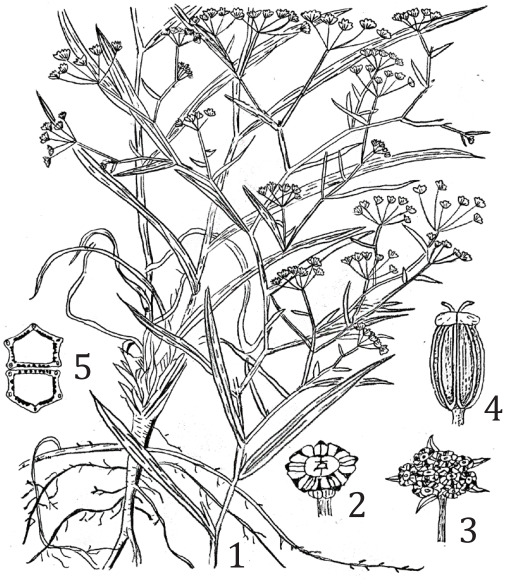
3.5
final sample
sample for the test required in this document

[SOURCE: ISO 21316:2019, 3.3, modified — Note to entry removed.]

4 Description

The structures of *Bupleurum chinense* DC., *Bupleurum scorzonerifolium* Willd., *Bupleurum falcatum* L. and the dried root are shown in Figure 1.

a) Plant of *Bupleurum chinense* DC. **b) *Bupleurum chinense* root**



c) Plant of *Bupleurum scorzonerifolium* Willd.

d) *Bupleurum scorzonerifolium* root

e) Plant of *Bupleurum falcatum* L.

f) *Bupleurum falcatum* root

Key

1 flowering branch
2 flower
3 inflorescence
4 fruit
5 cross-section of fruit
6 stamen
7 sepal

Figure 1 — Structures of *Bupleurum chinense, Bupleurum scorzonerifolium* and *Bupleurum falcatum* root

5 Requirements

5.1 General characteristics

The following requirements shall be met before sampling:

a) *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root materials shall be clean and dry.

b) The presence of living insects, mouldy root and external contaminants which are visible to the naked eye shall not be permitted.

c) The shape of the root shall be cylindrical or long and conical.

d) The root mass and the number of the residue shall be measured by electronic balance and counting.

e) The outer surface shall be brown with intermittent longitudinal wrinkles.

5.2 Macroscopic characteristics

5.2.1 *Bupleurum chinense*

The whole root is cylindrical or elongated conical, branched in the lower part, 6 cm to 15 cm in length and 0,3 cm to 0,8 cm in diameter. The upper part consists of a bulgy root crown, usually composed of 3 to 15 stem bases as well as the fibrous remnants of the leaf bases. The outer surface is blackish-brown or light brown, marked with longitudinal wrinkles and showing rootlet scars and lenticel-like protuberances. The texture is hard and compact, and difficult to break. The fracture shows concentric fibrous rings in the wood; the bark is thin, light brown or orange-brown, while the wood is whitish-yellow.

5.2.2 *Bupleurum scorzonerifolium*

The whole root is thinner than that of *Bupleurum chinense*, elongated conical, usually unbranched or very slightly branched in the lower part, up to 15 cm long and 0,3 cm to 0,5 cm in diameter. The root crown bears numerous fibres from the bases of wilted leaves in a brush-like shape. The outer surface is blackish-brown or reddish-brown and shows numerous annular striations near the root crown. The texture is slightly soft and the root breaks easily. The fracture is even and non-fibrous.

5.2.3 *Bupleurum falcatum*

The whole root is a long conical or column shape, single or branched, 10 cm to 15 cm in length and 0,5 cm to 1,5 cm in diameter. The upper part is thick and the lower part thin. The apex has numerous hairy fibres from withered leaves. The external surface is pale brown to brown with deep wrinkles. The texture is easily broken and the fractured surface is somewhat fibrous.

5.3 Moisture

The mass fraction of moisture should not be more than 10,0 %.

5.4 Total ash

The mass fraction of ash should not be more than 9,0 %.

5.5 Acid-insoluble ash

The mass fraction of acid insoluble ash should not be more than 3,5 %.

5.6 Thin-layer chromatogram (TLC) identification

The identification of marker compound, such as saikosaponin a, with TLC shall present the spots or bands obtained from the test and reference drug solution in the same position with the same colour.

5.7 Toxic adulterants of *Bupleurum longiradiatum* root

The characteristic fingerprint for the toxic adulterants of *Bupleurum longiradiatum* root should be determined.

5.8 Ethanol-soluble extractives

The mass fraction of ethanol-soluble extracts should not be less than 11,0 %.

5.9 Content of marker compounds

The content of marker compounds such as saikosaponin a and d shall be determined.

5.10 Heavy metals

The content of heavy metals such as arsenic, mercury, lead and cadmium shall be determined.

5.11 Pesticide residues

The content of pesticide residues such as benzene hexachloride, DDT and pentachloronitrobenzene shall be determined.

5.12 Sulfur dioxide residues

The content of sulfur dioxide residues should be determined.

6 Sampling

Sampling of *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root shall be carried out in accordance with ISO 23723.

7 Test methods

7.1 Macroscopic identification

Samples of not less than 500 g are taken from each batch randomly and observed with the naked eye, smelled and tasted.

7.2 Determination of moisture content

See Annex A for additional information.

7.3 Determination of total ash and acid-insoluble ash content

See Annex B for additional information.

7.4 Determination of ethanol-soluble extractives content

See Annex C for additional information.

7.5 Thin-layer chromatogram (TLC) identification

See Annex D for additional information.

7.6 Identification of the toxic adulterants of *Bupleurum longiradiatum* root

See Annex E for additional information.

7.7 Determination of marker compound(s)

See Annex F for additional information.

7.8 Determination of heavy metals

The testing method specified in ISO 18664 applies.

7.9 Determination of pesticide residues

The testing method specified in ISO 22258 applies.

7.10 Determination of sulfur dioxide residues

The testing method specified in ISO 22590 applies.

8 Test report

For each test method, the test report shall specify the following:

a) all information necessary for the complete identification of the sample;

b) the sampling method used;

c) the test method used, with reference to this document;

d) the test result(s) obtained;

e) all operating details not specified in this document, or regarded as optional, together with details of any incidents which could have influenced the test result(s);

f) any unusual features (anomalies) observed during the test;

g) the date of the test.

9 Packaging, storage and transportation

Packaging should not transmit any odour or flavour to the product and shall not contain substances which can damage the product or constitute a health risk. The packaging shall be strong enough to withstand normal handling and transportation.

The product shall be sealed and stored in a dry, shady and cool place. The storage temperature should be no higher than 25 °C.

The *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root shall be protected from light, moisture, pollution and entry of foreign substances during long-distance delivery.

10 Marking and labelling

The method specified in ISO 21371 shall apply. The following items shall be labelled on the packages:

a) product name;

b) category of the product in the marketed country or region;

c) net mass or quantity;

d) contact information;

e) name of raw materials;

e) date of production and expiry date of the products;

f) storage method;

g) batch or lot number.

Annex A
(informative)

Determination of moisture content

A.1 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

A.1.1 Sieve, sieve hole diameter (average): 850 µm ± 29 µm, 24 mesh.

A.1.2 Flat weighing bottle, 35 × 25 mm.

A.1.3 Analytical balance, weighing accuracy 0,01 mg.

A.1.4 Constant temperature blast oven, variable temperature range 30 °C to 300 °C, temperature control accuracy of ± 1 °C.

A.2 Sample analysis

Weigh accurately 2,0 g of the coarse powder (pass through a 24-mesh sieve), transfer to a dry-to-constant-mass flat weighing bottle. Open the bottle and dry at 105 °C for 5 h in a dryer. Cover the bottle, move out of the dryer, leave to cool for 30 min and weigh accurately. Dry at 105 °C for 1 h, leave to cool, then weigh accurately until the difference between two consecutive weighings is no more than 5 mg.

A.3 Record the result

According to the loss in mass, calculate the water content of herbs as a mass fraction (%).

Annex B
(informative)

Determination of total ash and acid-insoluble ash content

B.1 Reagents

Use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

B.1.1 Dilute HCl, take 234 ml hydrochloric acid (mass fraction: 36 % to 38 %), dilute with water to 1 000 ml. The liquid containing HCl should be 9,5 % to 10,5 %.

B.1.2 $AgNO_3$ reagent, desirable silver nitrate titration solution (0,1 mol/l).

B.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

B.2.1 Sieve, sieve hole diameter (average): 850 µm ± 29 µm, 24 mesh.

B.2.2 Analytical balance, weighing accuracy 0,01 mg.

B.2.3 Porcelain crucible, 1 200 °C high-temperature-resistant.

B.2.4 Muffle furnace, variable temperature range 200 °C to 600 °C, temperature control accuracy of ± 1 °C.

B.3 Sample analysis

B.3.1 Total ash

Weigh accurately 4,0 g of the coarse powder (pass through a 24-mesh sieve) in the porcelain crucible, heat slowly, with a gradual increase in temperature to 500 °C to 600 °C, taking care to avoid burning, to complete carbonization and constant mass.

B.3.2 Acid-insoluble ash

Take the ash obtained on the item and carefully add about 10 ml of dilute HCl to the crucible. Cover the crucible with a watch glass, place in a water bath and heat for 10 min. Wash the surface dish with 5 ml of hot water, wash liquor into the crucible, filter with an ashless filter paper, wash the crucible residue with water onto the filter paper and wash until the lotion does not show significant chloride reaction. The filter cake, together with the filter paper, is transferred to the same crucible, dried and ignited to constant mass.

B.4 Record the result

Calculate the content of total ash and acid insoluble ash of the test sample as a mass fraction (%).

Annex C
(informative)

Determination of ethanol-soluble extractives content

C.1 Reagents

Use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

C.1.1 Water, distilled.

C.1.2 Ethanol, of recognized analytical grade.

C.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

C.2.1 Sieve, sieve hole diameter (average) 850 µm ± 29 µm, 24 mesh.

C.2.2 Analytical balance, weighing accuracy 0,01 mg.

C.2.3 Stopper conical flask, 250 ml.

C.2.4 Evaporation dish, 100 ml.

C.2.5 Constant temperature blast oven, variable temperature range 30 °C to 300 °C, temperature control accuracy of ± 1 °C.

C.3 Sample analysis

Ethanol-soluble extractives content in samples can be determined by the hot-dip method.

Determination can be conducted via the following steps:

a) Weigh approximately 2,0 g of the powder into a 250 ml stopper conical flask. Accurately add 50 ml ethanol:water (volume ratio 95:5). Weigh and allow to stand for 1 h.

b) Heat under reflux to slightly boil on a water bath for 1 h. Cool and weigh again. Replenish the loss in mass with water, mix well and filter.

c) Weigh a dried evaporating dish. Transfer 25 ml of the successive filtrate into an evaporating dish. Evaporate the filtrate to dryness on a water bath.

d) Calculate the percentage of ethanol-soluble extractives on the dried basis (%).

C.4 Record the result

Calculate the percentage of ethanol-soluble extractives on the dried basis as a mass fraction (%).

Annex D
(informative)

Thin-layer chromatogram (TLC) identification

D.1 Reagents

Use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

D.1.1 Water, distilled.

D.1.2 Ethyl acetate, ethanol, anhydrous methanol, analytical grade.

D.1.3 2 % of dimethylaminobenzaldehyde in 40 % sulfuric acid solution, 1 g p-dimethylamino-benzaldehyde dissolved in 50 ml 40 % sulfuric acid solution.

D.1.4 Saikosaponin a and d reference substance, purity ≥ 98 %.

D.1.5 Prefabricated thin layer high-efficiency silica gel G plate, 100 × 200 mm high-efficiency silica gel G plate, activated at 105 °C for half an hour before use.

D.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

D.2.1 Sieve, sieve hole diameter (average) 850 µm ± 29 µm, 24 mesh.

D.2.2 Analytical balance, weighing accuracy 0,01 mg.

D.2.3 Centrifuge tube, 50 ml.

D.2.4 Constant temperature blast oven, variable temperature range 30 °C to 300 °C, temperature control accuracy of ± 1 °C.

D.2.5 UV detection lights, 365 nm.

D.3 Sample analysis

D.3.1 Preparation of reference standards solution

Dissolve reference standards of saikosaponin a and d in methanol to prepare the reference standards solution of 0,5 mg/ml. Store at −20 °C before use.

D.3.2 Preparation of the test solution

Weigh approximately 0,5 g of the powder into a 50 ml centrifuge tube. Add 20 ml anhydrous methanol and sonicate for 10 min, then filter. Take the layer of filtrate to dryness. Dissolve the residue with 5 ml of methanol as the test solution.

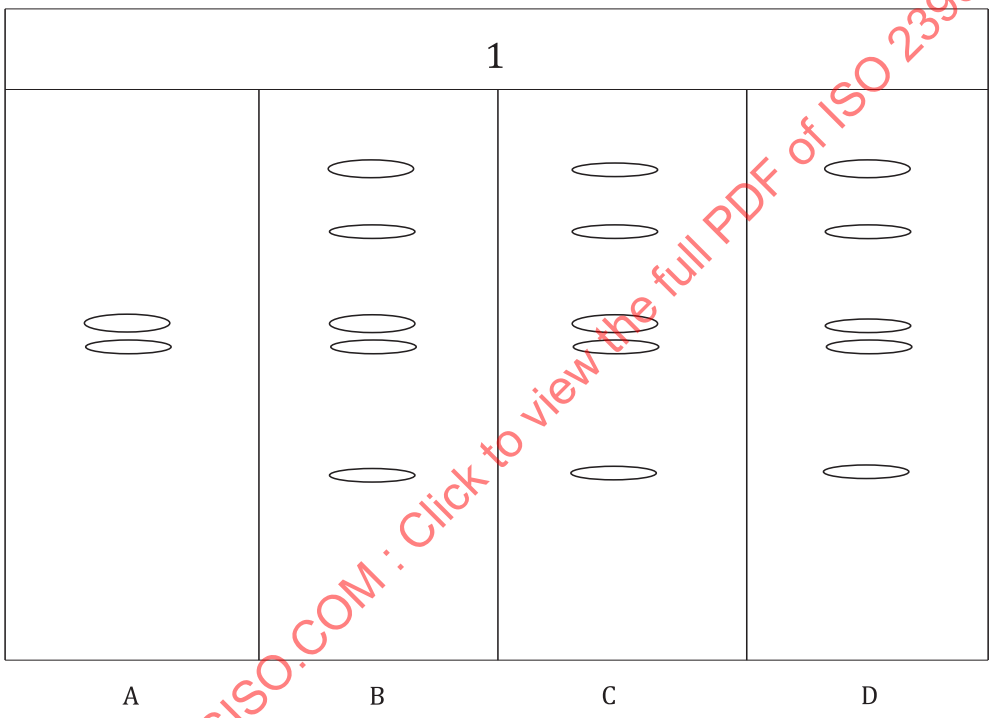
D.3.3 Identification by TLC

Apply 5 µl of the reference standard solution and 5 µl of the test solution on identical TLC plates (silica gel) previously dried at 105 °C for 30 min in the oven. Develop with a solution of the mixture of ethyl acetate, ethanol and water (volume ratio 8:2:1). Take the plate out and dry in air. Spray 2 % solution of p-dimethylaminobenzaldehyde in 40 % solution of sulfuric acid over the TLC plate and heat at 60 °C until the colour looks clear. Identify the saikosaponin spots of test solution by comparing the positions and colours with those of the reference standard solution.

D.4 Record the result

In the same TLC chromatogram of the test solution, there are spots or fluorescent spots of the same colour at the positions corresponding to the standards solution.

Typical reference TLC chromatograms are shown in Figure D.1.



Key

1 top of the plate
A reference standard solution saikosaponin d and saikosaponin a (from top to bottom)
B *Bupleurum chinense* root
C *Bupleurum scorzonerifolium* root
D *Bupleurum falcatum* root

Figure D.1 — Schematic diagram of typical TLC chromatograms of *Bupleurum chinense*, *Bupleurum scorzonerifolium* and *Bupleurum falcatum* root

Annex E
(informative)

Identification of the toxic adulterants of *Bupleurum longiradiatum* root

E.1 Reagents

Use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

E.1.1 Aqueous ammonia, anhydrous methanol, analytical grade.

E.1.2 Water, distilled.

E.1.3 Saikosaponin a, d reference substance, for content analysis, purity ≥ 98 %.

E.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

E.2.1 Sieve, sieve hole diameter (average) 250 µm ± 9,9 µm, 65 mesh.

E.2.2 Round-bottomed flask, 100 ml.

E.2.3 Analytical balance, weighing accuracy 0,01 mg.

E.2.4 Ultrasound, power 200 W, frequency 40 kHz.

E.2.5 HPLC-UV system.

E.3 Sample analysis

E.3.1 Chromatographic conditions and system applicability test

Use octadecylsilane chemically bonded silica as a filler. Use acetonitrile as mobile phase A and water as mobile phase B. Gradient elution is performed according to Table E.1. The detection wavelength is 210 nm and the flow rate is 0,8 ml/min. The standard deviation of the peak area of saikosaponin d should be no greater than 5,0 % and the theoretical plate number of saikosaponin a should be no less than 10 000.

Table E.1 — Mixture of mobile phases A and B in gradient elution

Time min	Mobile phase A %	Mobile phase B %
0 to 30	30→50	70→50
30 to 40	50→75	50→25
40 to 50	75→90	25→10

Table E.1 *(continued)*

Time min	Mobile phase A %	Mobile phase B %
50 to 65	90→100	10→0
65 to 70	100	0

E.3.2 Preparation of reference standards solution

Dissolve reference standards of saikosaponin a and d in methanol to prepare the reference standards solution of 0,1 mg/ml.

E.3.3 Preparation of the test solution

Weigh accurately 0,25 g of the powder into a 50-ml centrifuge tube. Add accurately 20,0 ml 25 % volume fraction of ammonia-methanol solvent and sonicate for 1 h, centrifuge for 10 min (3 200 × *g*) then filter with methanol solvent. Take the layer of filtrate to dryness. Dissolve the residue in methanol. Transfer the solution to a 5-ml volumetric flask and make up to the mark with methanol, then filter with 0,45 µm microporous membrane (polytetrafluoroethylene) as the test solution.

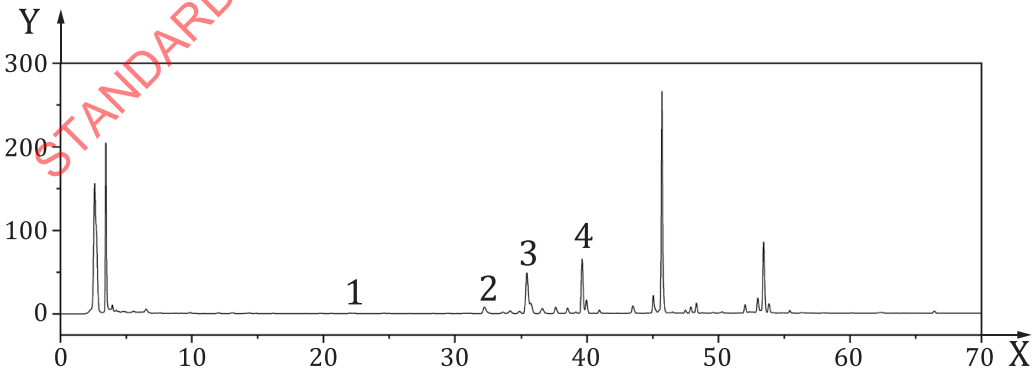
E.3.4 Assay

Inject 10 µl reference working solution and 10 µl test solution into the HPLC and determine the peak areas and chromatograms.

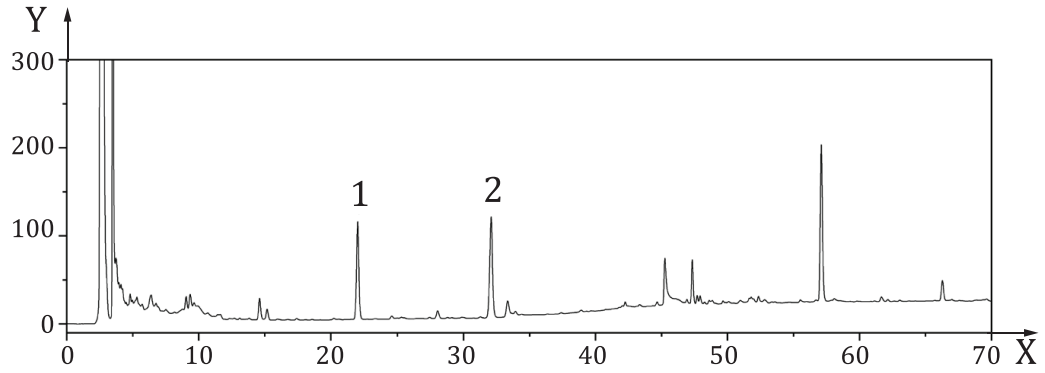
E.4 Record the result

Caution should be applied as the adulterant *Bupleurum longiradiatum* root is toxic and can result in vomiting, convulsions, syncope or even death if misused as a substitute for Bupleuri Radix[11].

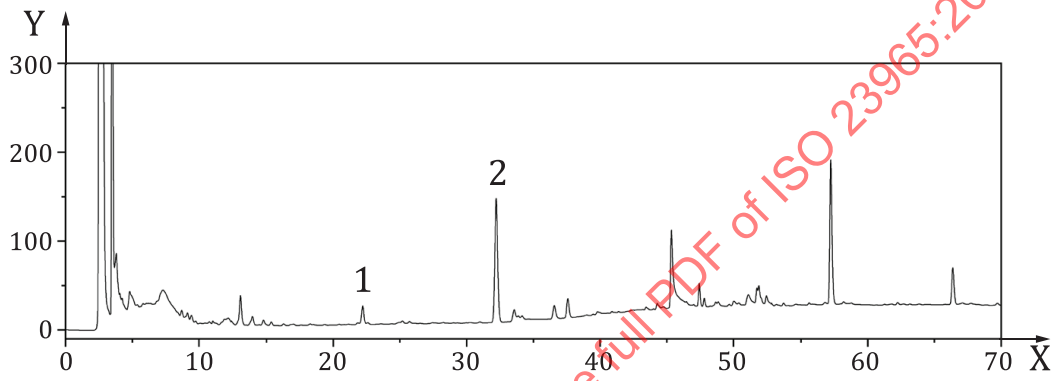
The relative deviation of retention time of saikosaponin d (peak 2) should not be greater than 5 %. Under the same liquid chromatographic conditions, the retention time of the characteristic peaks in the test solutions are compared with saikosaponin d to calculate relative retention time. The chromatogram of test solutions should contain saikosaponin a (peak 1, relative retention time: 0,69, variable range: ± 0,03) and saikosaponin d (relative retention time: 1,00). If the spectra of test solutions contain the characteristic peak 3 (relative retention time: 1,09, variable range: ± 0,03) or peak 4 (relative retention time: 1,23, variable range: ± 0,03), the test samples can be considered as (or mixed with) the toxic adulterants of *Bupleurum longiradiatum* root (Figure E.1)



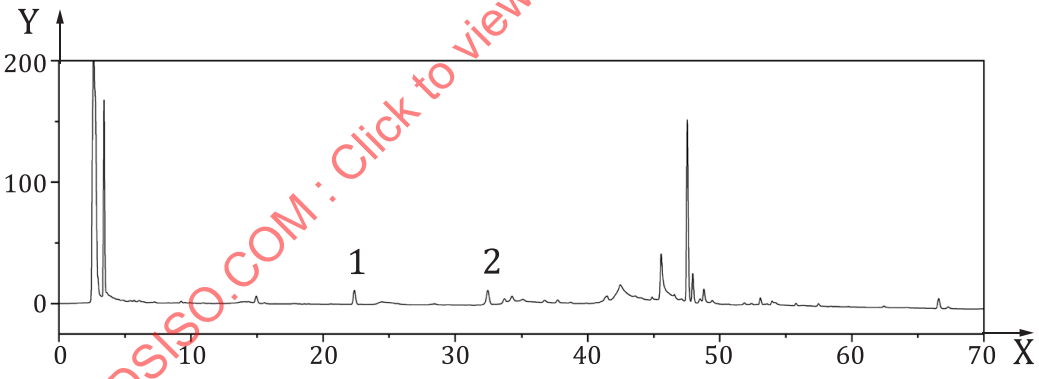
a) Chromatogram of *Bupleurum longiradiatum* root



b) Chromatogram of *Bupleurum chinense* root



c) Chromatogram of *Bupleurum scorzonerifolium* root



d) Chromatogram of *bupleurum falcatum* root

Key

X min
Y mAU
1 saikosaponin a
2 saikosaponin d
3, 4 characteristic peaks of *bupleurum longiradiatum*

Figure E.1 — Characteristic chromatograms of Bupleuri Radix

Annex F
(informative)

Determination of marker compounds

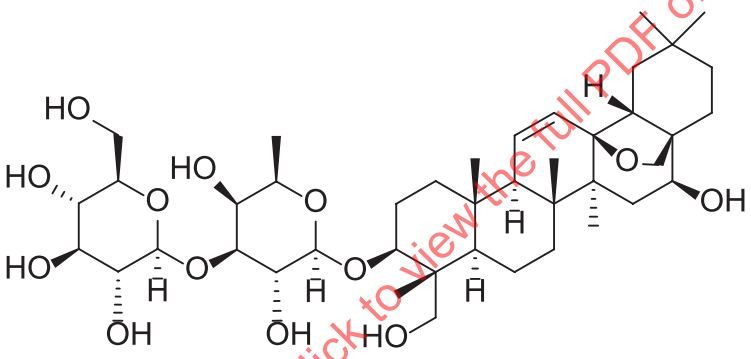
F.1 Reagents

Use only reagents of recognized analytical grade and only distilled water or water of equivalent purity.

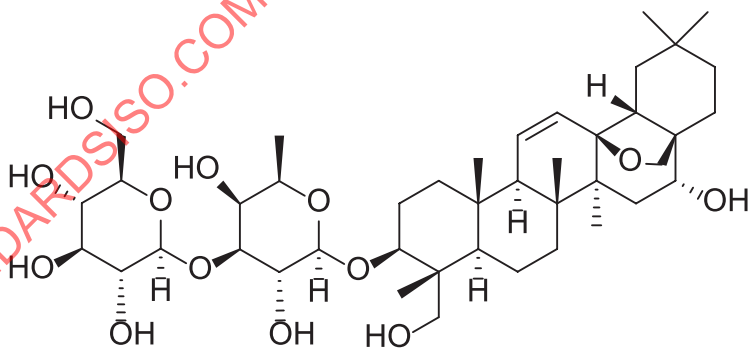
F.1.1 **Ammonia, anhydrous methanol**, analytical grade.

F.1.2 **Methanol, acetonitrile**, HPLC grade.

F.1.3 **Saikosaponins a, d reference standards**, for content analysis, purity ≥ 98 % (Figure F.1).



a) Saikosaponin a



b) Saikosaponin d

Figure F.1 — Chemical structures of saikosaponins

F.2 Apparatus

The usual laboratory apparatus and, in particular, the following shall be used:

F.2.1 **Sieve**, sieve hole diameter (average): 250 µm ± 9,9 µm, 65 mesh.