
**Liquorice extracts (*Glycyrrhiza glabra* L.) —
Determination of glycyrrhizic acid
content — Method using high-performance
liquid chromatography**

*Extraits de réglisse (*Glycyrrhiza glabra* L.) — Détermination de la teneur en
acide glycyrrhizique — Méthode par chromatographie liquide à haute
performance*



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Liquorice extracts (*Glycyrrhiza glabra* L.) — Determination of glycyrrhizic acid content — Method using high-performance liquid chromatography

1 Scope

This International Standard describes a method for determining the glycyrrhizic acid content of liquorice extract (*Glycyrrhiza glabra* L.) by high-performance liquid chromatography.

The method is not applicable to raw or ground liquorice root.

2 Principle

The sample and standard solutions are prepared, then the glycyrrhizic acid content is determined by high-performance liquid chromatography using the method described in this International Standard.

3 Reagents

Use only reagents of recognized analytical grade, unless otherwise specified.

3.1 Water, HPLC grade.

3.2 Reference substance, monoammoniacal glycyrrhizate (GMA).

If a reference material of guaranteed purity is not available, it is recommended that the users of this International Standard come to an agreement between the interested parties on the purity of the reference substance.

3.3 Acetonitrile, HPLC grade.

3.4 Acetic acid, analytical grade.

3.5 Elution solvent (mobile phase), composed of the following:

- 38 volumes acetonitrile (3.3),
- 61 volumes water (3.1),
- 1 volume acetic acid (3.4).

Using the measuring cylinder (4.3), prepare the elution solvent as follows.

Mix 1 volume of acetic acid with 61 volumes of water, then filter the mixture through a filter for aqueous solvents (4.5).

Filter 38 volumes of acetonitrile through a filter for organic solvents (4.4).

Add the filtered acetonitrile to the filtered water/acetic acid mixture. Mix, then degas the elution solvent in the ultrasound cell (4.8.3) or any suitable system.

Do not keep this solvent more than 48 h at ambient temperature.

4 Apparatus

Usual laboratory apparatus and, in particular, the following.

- 4.1 **Pipettes**, of capacities 5 ml and 10 ml.
- 4.2 **Volumetric flasks**, of capacities 50 ml and 100 ml.
- 4.3 **Measuring cylinder**.
- 4.4 **Filter for organic solvents**, 0,5 µm pore size.
- 4.5 **Filter for aqueous solvents**, 0,45 µm pore size.
- 4.6 **Oven**, capable of being maintained at 105 °C ± 2 °C.
- 4.7 **Analytical balance**, capable of weighing to the nearest 0,000 1 g.
- 4.8 **Separation system**, as follows.
 - 4.8.1 **Chromatograph**, high-performance liquid phase.
 - 4.8.2 **Pumping system**, for obtaining and maintaining a constant or programmed high pressure flow.
 - 4.8.3 **Solvent degassing system**, such as an ultra-sound cell or any suitable system.
 - 4.8.4 **Ultraviolet detection system**, adjustable to a wavelength of 254 nm.
- 4.9 **Recorder or integrator**, of compatible performance with all the apparatus.
- 4.10 **Column**, as follows:
 - material: stainless steel or glass;
 - length: 10 cm to 25 cm;
 - internal diameter: 0,4 cm to 0,5 cm;
 - stationary phase: bonded phase silica with octadecyl C18 derived functional group, maximum particle size 5 µm;
 - column efficiency: the number recommended of theoretical plates is 7 000 to 10 000.
- 4.11 **Pre-column**, as follows:
 - material: stainless steel;
 - length: 10 mm or 25 mm;
 - internal diameter: 2 mm or 4 mm;
 - stationary phase: bonded phase silica with octadecyl C18 derived functional group

5 Procedure

5.1 Preparation of standard solutions

5.1.1 Preparation of the 0,5 mg/ml solution

In a 100 ml volumetric flask (4.2) weigh, to the nearest 10^{-4} g, 50 mg of monoammoniacal glycyrrhizate (3.2).

Add the elution solvent (3.5) and dissolve the monoammoniacal glycyrrhizate (3.2). Make up to the mark with a further quantity of elution solvent.

Filter if necessary through the organic solvent filter (4.4).

It is essential to prepare a new solution every day.

5.1.2 Preparation of dilute solutions containing 0,05 mg/ml, 0,075 mg/ml and 0,1 mg/ml

5.1.2.1 0,05 mg/ml standard solution

Using a pipette (4.1), transfer 5 ml of the 0,5 mg/ml standard solution (5.1.1) to a 50 ml volumetric flask (4.2). Make up to the mark with the elution solvent (3.5).

5.1.2.2 0,075 mg/ml standard solution

Using a pipette (4.1), transfer 15 ml of the 0,5 mg/ml standard solution (5.1.1) to a 100 ml volumetric flask (4.2). Make up to the mark with the elution solvent (3.5).

5.1.2.3 0,1 mg/ml standard solution

Using a pipette (4.1), transfer 10 ml of the 0,5 mg/ml standard solution (5.1.1) to a 50 ml volumetric flask (4.2). Make up to the mark with the elution solvent (3.5).

It is essential to prepare a new solution every day.

5.2 Preparation of the solution for analysis

5.2.1 Prepare the solution for analysis such that the area of the glycyrrhizic acid peak obtained is between the peaks for the other two standard solutions, on the basis of the recommended masses and volumes specified in Table 1, according to the assumed glycyrrhizic acid content of the sample.

Table 1 — Examples of quantities and volumes to be used according to the assumed glycyrrhizic acid content of the liquorice extracts

Assumed glycyrrhizic acid content	Mass of test portion	Volume of flask	Volume of pipetted solution	Volume of flask
%	mg	V_1 ml	V_2 ml	V_3 ml
3 to 5	1	50	5	50
5 to 10	1	100	5	50
10 to 15	0,50	100	5	50
20 to 25	0,25	100	5	50

5.2.2 Weigh, to the nearest 10^{-4} g, a test portion of mass m_0 into a volumetric flask of volume V_1 ml.

Add an appropriate quantity of elution solvent (3.5) to dissolve the sample. Then make up to the mark with elution solvent.

Using a pipette, transfer a volume (V_2) of this solution to a volumetric flask of volume V_3 ml.

Add an appropriate quantity of elution solvent (3.5), mix and make up to the mark with elution solvent.

Filter this last solution through the filter for organic solvents (4.3) prior to injecting.

5.3 Determination of the water and volatile matter content of the sample

Weigh, to the nearest 10^{-4} g, a test portion (m_0) of exactly 2 g and place in a calibrated watchglass.

Place it in the oven (4.6) set at 105 °C for 24 h, until a constant mass is reached. Cool then weigh the residue (m_1).

The water and volatile matter content of the sample, w , expressed as a percentage by mass, is equal to:

$$w = \frac{(m_0 - m_1)}{m_0} \times 100 \%$$

where

m_0 is the mass of the test portion, in grams;

m_1 is the mass of the residue obtained, in grams.

6 Determination

6.1 Inject 10 μ l of solution 5.1.2.1.

6.2 Inject 10 μ l of solution 5.1.2.2.

6.3 Inject 10 μ l of solution 5.1.2.3.

6.4 Inject 10 μ l of the solution for analysis (5.2.2).

6.5 Carry out the operations described in 6.1 to 6.4 twice.

NOTE If the injection and detection systems allow this, it is possible to inject quantities from 5 μ l to 10 μ l.

6.6 Measure the area of the glycyrrhizic acid peak on the chromatograms obtained for the standard solutions (see annex A).

6.7 Plot a curve of glycyrrhizic acid peak area against the concentration of monoammoniacal glycyrrhizate in the standard solutions.

A calibration line passing through the origin is obtained.

6.8 Using the chromatogram for the sample solution, measure the area of the glycyrrhizic acid peak.

From the calibration line, read the monoammoniacal glycyrrhizate concentration, c , in milligrams per millilitre.

7 Expression of results

The glycyrrhizic acid content, w_G , expressed as a percentage by mass on dry matter, is calculated using the formula:

$$w_G = \frac{c \times V_1 \times V_3 \times 100 \times p \times 822}{m_0 \times V_2 (100 - w) \times 839}$$

where

m_0 is the mass of the test portion, in grams;

c is the mean value of the concentrations obtained, in milligrams per millilitre, of monoammoniacal glycyrrhizate read from the calibration line;

p is the purity of the reference substance, in percent;

w is the water and volatile matter content of the sample for analysis, as a percentage by mass, determined in 5.3;

822 is the molar mass of glycyrrhizic acid;

839 is the molar mass of monoammoniacal glycyrrhizate;

V_1, V_2, V_3 have the same meanings as indicated in Table 1.

8 Test report

The test report shall specify:

- all information necessary for the complete identification of the sample;
- the sampling method used, if known;
- the test method used, with reference to this International Standard;
- all operating details not specified in this International Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);
- the test result(s) obtained; or
- if the repeatability has been checked, the final quoted result obtained.

Annex A
(informative)

Examples of chromatograms

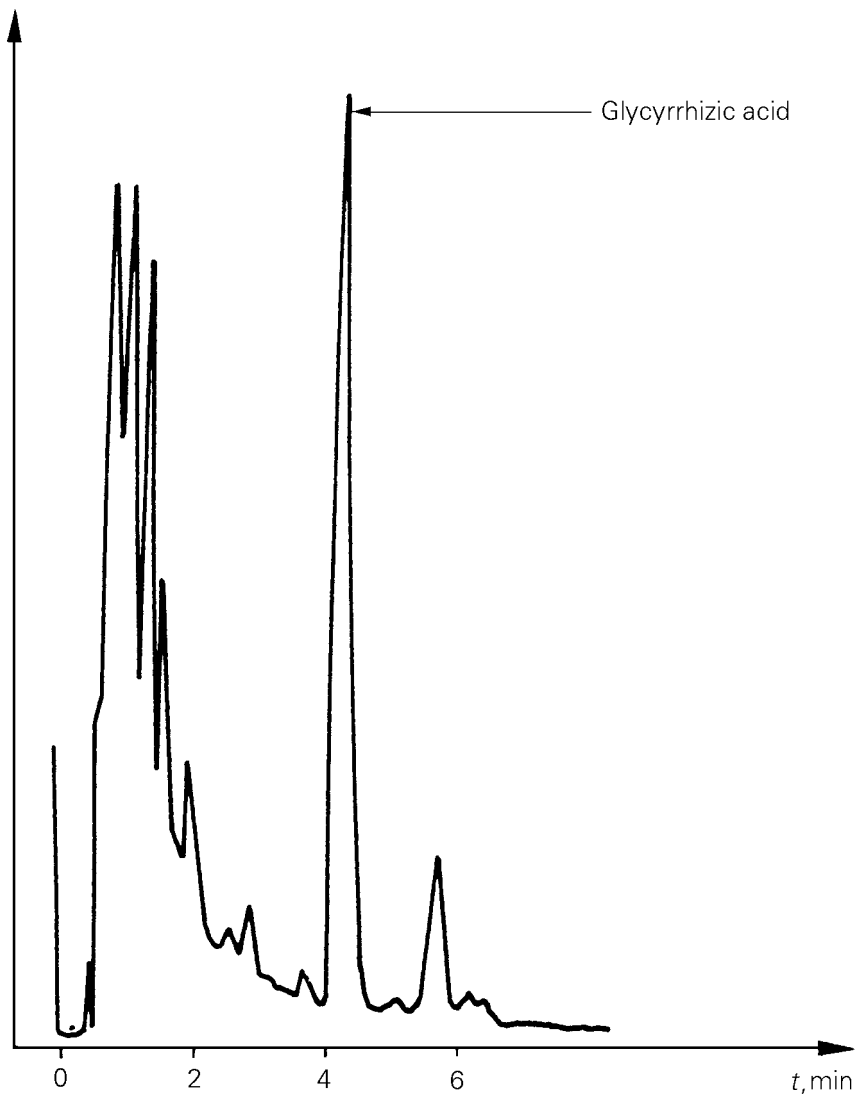


Figure A.1 — Chromatogram of a liquorice extract

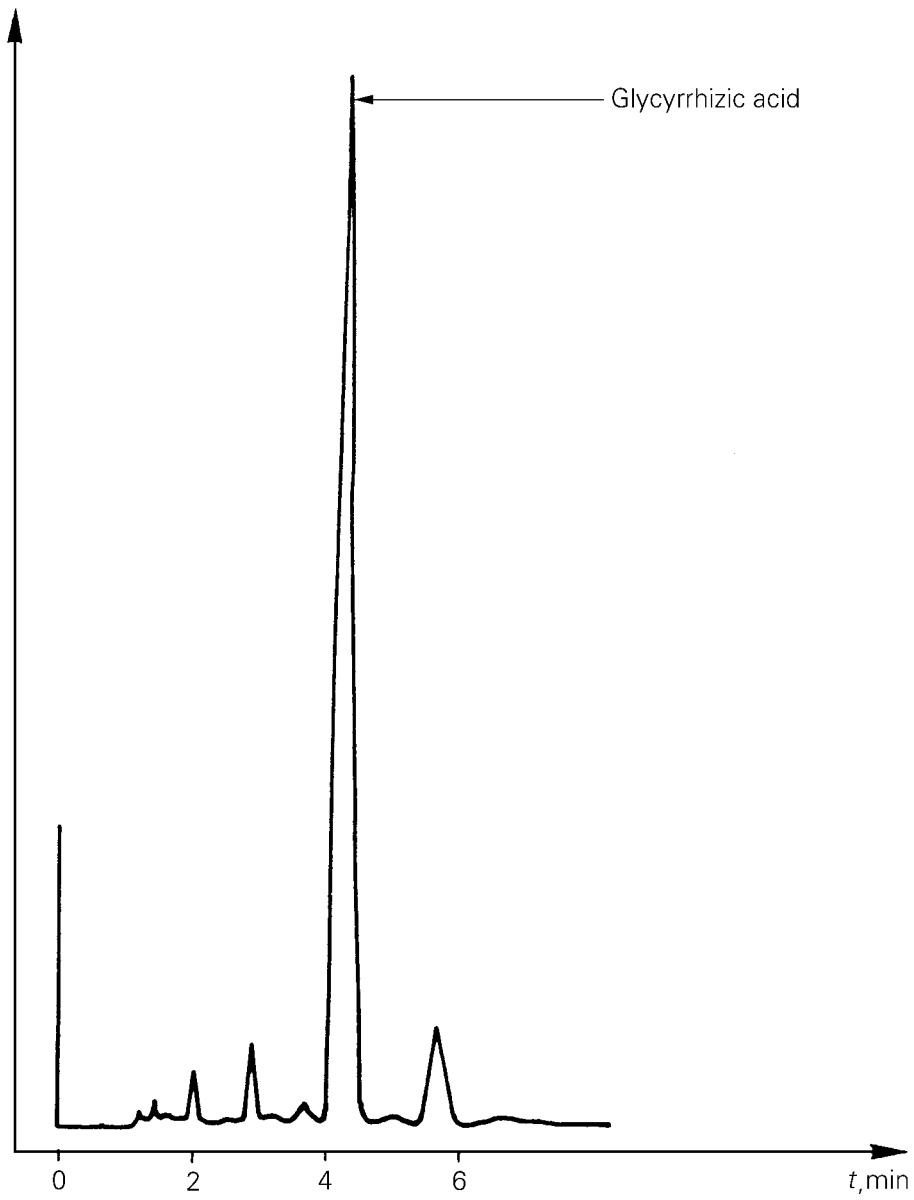


Figure A.2 — Chromatogram of the reference substance

Bibliography

- [1] ISO 8432, *Essential oils — Analysis by high performance liquid chromatography — General method*.

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