

---

# International Standard



# 5558

---

INTERNATIONAL ORGANIZATION FOR STANDARDIZATION • МЕЖДУНАРОДНАЯ ОРГАНИЗАЦИЯ ПО СТАНДАРТИЗАЦИИ • ORGANISATION INTERNATIONALE DE NORMALISATION

---

## **Animal and vegetable fats and oils — Detection and identification of antioxidants — Thin-layer chromatographic method**

*Corps gras d'origines animale et végétale — Recherche et identification des antioxygènes — Méthode par chromatographie en couche mince*

First edition — 1982-10-01

**(standards.iteh.ai)**

[ISO 5558:1982](https://standards.iteh.ai/catalog/standards/sist/13e68ccc-6cd3-4933-a9f5-b4ded54db1dc/iso-5558-1982)

<https://standards.iteh.ai/catalog/standards/sist/13e68ccc-6cd3-4933-a9f5-b4ded54db1dc/iso-5558-1982>

---

UDC 664.3 : 543.544 : 547.53

Ref. No. ISO 5558-1982 (E)

Descriptors : agricultural products, animal fats, vegetable fats, detection, antioxidants, chromatographic analysis.

## Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards institutes (ISO member bodies). The work of developing International Standards is carried out through ISO technical committees. Every member body interested in a subject for which a technical committee has been set up 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.

Draft International Standards adopted by the technical committees are circulated to the member bodies for approval before their acceptance as International Standards by the ISO Council.

International Standard ISO 5558 was developed by Technical Committee ISO/TC 34, *Agricultural food products*, and was circulated to the member bodies in March 1981.

It has been approved by the member bodies of the following countries :

Australia	Hungary	Portugal
Austria	India	Romania
Brazil	Iraq	South Africa, Rep. of
Canada	Israel	Spain
Czechoslovakia	Italy	Sri Lanka
Dominican Republic	Korea, Rep. of	Tanzania
Egypt, Arab Rep. of	Mexico	Thailand
Ethiopia	Netherlands	United Kingdom
France	New Zealand	USSR
Germany, F.R.	Philippines	Yugoslavia

No member body expressed disapproval of the document.

This International Standard has also been approved by the International Union of Pure and Applied Chemistry (IUPAC).

# Animal and vegetable fats and oils — Detection and identification of antioxidants — Thin-layer chromatographic method

## 1 Scope and field of application

This International Standard specifies a thin-layer chromatographic method for the detection and identification of the following antioxidants in animal and vegetable fats and oils :

- nordihydroguaiaretic acid (NDGA)
- propylgallate (PG)
- octylgallate (OG)
- dodecylgallate (DG)
- tertibutylhydroquinone (TBHQ)
- butylhydroxyanisole (*tert*-butyl-4-methoxyphenol) (BHA)
- $\alpha$ -tocopherol
- butylhydroxytoluene (2,6-di-*tert*-butyl-4-methylphenol) (BHT)

The antioxidants are listed in the order of their  $R_f$  values.

The detection of  $\alpha$ -tocopherol is sometimes difficult as the spots of this antioxidant are of a generally diffuse aspect.

The method has limits of detection of 20 mg/kg (ppm) for BHT, and 10 mg/kg (ppm) for the other antioxidants. It should be noted that BHT is not completely isolated by this method.

## 2 Principle

Dissolution of a test portion in *n*-hexane and extraction of the antioxidants with acetonitrile.

Identification of the antioxidants by thin-layer chromatography.

## 3 Reagents

All reagents shall be of recognized analytical quality. The water used shall be distilled water or water of at least equivalent purity.

**3.1 Silica powder with binder**, thin-layer chromatographic quality, of particle size less than 30  $\mu\text{m}$ .

**3.2 Methanol**, containing not more than 0,5 % (*m/m*) of water.

**3.3 Ethanol**, 96 % (*V/V*).

**3.4 *n*-hexane** or, failing this, **light petroleum** having a distillation range from 30 to 60 °C.

**3.5 Acetonitrile saturated with *n*-hexane.**

Pour 900 ml of acetonitrile into a 1 000 ml flask and add 100 ml of the *n*-hexane (3.4) and a little anhydrous sodium sulphate or anhydrous calcium chloride. Shake, stopper the flask and allow to stand overnight.

**3.6 *n*-hexane saturated with acetonitrile.**

Pour 900 ml of the *n*-hexane (3.4) into a 1 000 ml flask and add 100 ml of acetonitrile and a little anhydrous sodium sulphate or anhydrous calcium chloride. Shake, stopper the flask and allow to stand overnight.

**3.7 Indicator solution** : 2,6-dichloro-*p*-benzoquinone-4-chloroimide, 10 g/l solution in 96 % (*V/V*) ethanol.

Prepare, just before use, by dissolving 500 mg of 2,6-dichloro-*p*-benzoquinone-4-chloroimide in 50 ml of the ethanol (3.3).

**3.8 Developing solvent mixture.**

Prepare, just before use, a mixture of two volumes of the *n*-hexane (3.4), two volumes of benzene and one volume of glacial acetic acid.

**WARNING** — Benzene is toxic and flammable and care should be exercised in using it.

**3.9 Antioxidant standard solutions**, containing 1 g of antioxidant per litre of methanol.

In a series of eight beakers, dissolve separately 100 mg of each of the antioxidants (see clause 1) in the methanol (3.2), transfer to a series of eight 100 ml volumetric flasks, make up to the mark with the methanol and mix.

iTeh STANDARD PREVIEW  
(standards.iteh.ai)

ISO 5558:1982  
<https://standards.iteh.ai/catalog/standards/sist/933-a9f5-b4ded54db1dc/iso-5558-1982>

## 4 Apparatus

Usual laboratory equipment, and in particular :

**4.1 Developing tank for thin-layer chromatography**, made of glass, suitable for glass plates of dimensions 200 mm × 200 mm, fitted with a ground glass lid.

**4.2 Spreader and platform**, for preparation of the plates.

**4.3 Glass plates**, 200 mm × 200 mm, coated with a layer of silica gel of thickness 0,40 mm.

Use commercially available prepared plates, or prepare as follows.

Clean the plates thoroughly with ethanol, *n*-hexane and acetone until all traces of fat are removed.

Weigh 30 g of the silica gel (3.1) into a 250 ml conical flask (4.8) and add 60 ml of water. Stopper the flask and thoroughly mix the contents by shaking vigorously for 1 min.

Immediately apply a 0,4 mm layer of the silica gel slurry on to the surface of the plates using the spreader (4.2).

Dry the plates at ambient temperature for 15 min, and then place them in the oven (4.6), controlled at  $103 \pm 2$  °C, for 1 h. Allow to cool in the dessicator (4.13) and store in the dessicator until required.

**4.4 Rotary evaporator**.

**4.5 Separating funnels**, of capacity 250 ml.

**4.6 Electrically heated drying oven**, preferably well ventilated, capable of being controlled at  $103 \pm 2$  °C.

**4.7 Electrically heated drying oven**, preferably well ventilated, capable of being controlled at  $60 \pm 2$  °C.

**4.8 Conical flask**, of capacity 250 ml, with a ground glass stopper.

**4.9 Volumetric flasks**, of capacity 100 ml, with ground necks and ground glass stoppers.

**4.10 Beaker**, tall form, of capacity 150 ml.

**4.11 Round-bottomed flask**, of capacity 250 ml, suitable for use with the rotary evaporator (4.4).

**4.12 Syringe**, of capacity 20 µl, graduated in microlitres.

**4.13 Desiccator**.

**4.14 Apparatus for spraying the indicator solution on to the plates**.

## 5 Procedure

### 5.1 Test portion

Take a test portion of 7,5 to 10 g from the laboratory sample.

### 5.2 Extraction of antioxidants

Dissolve the test portion in 100 ml of the *n*-hexane (3.4) in the 150 ml beaker (4.10), heating gently, if necessary, and transfer the solution into one of the 250 ml separating funnels (4.5), taking care that no insoluble particles pass into the separating funnel.

Rinse the beaker with 25 ml of the *n*-hexane (3.4) and transfer the rinsing liquid into the separating funnel, again taking care that no insoluble particles pass into the separating funnel.

Add 25 ml of the acetonitrile saturated with *n*-hexane (3.5) and shake for 1 min. Collect the acetonitrile phase (lower layer) in a second 250 ml separating funnel (4.5).

NOTE — If an emulsion forms, turn the separating funnel carefully under a stream of water at about 50 °C until clear phases are obtained.

Carry out three further extractions of the *n*-hexane phase using 25 ml of acetonitrile saturated with *n*-hexane (3.5) each time.

Combine the acetonitrile extracts and wash twice using 25 ml of the *n*-hexane saturated with acetonitrile (3.6) each time.

Transfer the acetonitrile phase to the 250 ml round-bottomed flask (4.11) and evaporate the solvent under reduced pressure in the rotary evaporator (4.4) at as low a temperature as possible. It is essential that the temperature of the water bath does not exceed 40 °C.

Dissolve the residue in 2 ml of the methanol (3.2) and pour the solution into a small flask. If the residue is not completely dissolved, filter the solution.

NOTE — If an antioxidant content of less than 100 mg/kg (ppm) is expected, dissolution of the residue in only 1 ml of methanol is recommended.

### 5.3 Chromatography and detection

Coat the walls of the developing tank (4.1) with filter paper.

Pour the developing solvent mixture (3.8) into the developing tank to a depth of about 1 cm and put on the lid. Allow to stand in the dark for 1 to 2 h, in order to saturate the atmosphere inside the tank with the solvent vapours.

Using the syringe (4.12), transfer 10 µl and 20 µl portions of the methanolic solution of extracted antioxidants, prepared as described in 5.2, to two distinctive points 20 mm apart and 20 mm from the edge of the plate (4.3), previously activated for 1 h at a temperature of 60 °C in the oven (4.7). Transfer 4 µl of each of the antioxidant standard solutions (3.9) to distinctive points 20 mm apart and 20 mm from the same edge of the plate.

Mark a line parallel to the edge of the plate and 150 mm from the points of application. Place the plate in the developing tank and leave in the dark until the solvent front reaches the marked line.

Remove the plate from the tank and allow to dry under a fume hood.

Spray the indicator solution (3.7) on to the plate, using the apparatus (4.14), and place the plate in the oven (4.6), controlled at  $103 \pm 2$  °C, for 10 to 15 min.

Remove the plate from the oven, and compare the  $R_f$  values of the spots resulting from the standard solutions with those of the extracted antioxidants.

Further information on the identification of antioxidants can be obtained by placing the plate, for about 30 s, at laboratory temperature, in a developing tank saturated with ammonia and by comparing the resulting colours for the various antioxidants.

## 6 Test report

The test report shall show the method used and the results obtained. It shall also mention all operating conditions not specified in this International Standard, or regarded as optional, as well as any circumstances that may have influenced the results.

The test report shall include all the information necessary for the complete identification of the sample.

# iTeh STANDARD PREVIEW (standards.iteh.ai)

[ISO 5558:1982](#)

<https://standards.iteh.ai/catalog/standards/sist/13e68ccc-6cd3-4933-a9f5-b4ded54db1dc/iso-5558-1982>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

This page intentionally left blank

[ISO 5558:1982](#)

<https://standards.iteh.ai/catalog/standards/sist/13e68ccc-6cd3-4933-a9f5-b4ded54db1dc/iso-5558-1982>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

This page intentionally left blank

[ISO 5558:1982](#)

<https://standards.iteh.ai/catalog/standards/sist/13e68ccc-6cd3-4933-a9f5-b4ded54db1dc/iso-5558-1982>

**iTeh STANDARD PREVIEW**  
**(standards.iteh.ai)**

This page intentionally left blank

[ISO 5558:1982](#)

<https://standards.iteh.ai/catalog/standards/sist/13e68ccc-6cd3-4933-a9f5-b4ded54db1dc/iso-5558-1982>