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Animal and vegetable fats and oils — Determination of phthalates in vegetable oils

~~*Corps gras d'origine végétale et animale – Détermination des phtalates dans les huiles végétales*~~

FDIS stage

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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~~document 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~~www.iso.org/directives).

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This document was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils.*

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~~www.iso.org/members.html.

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Animal and vegetable fats and oils — Determination of phthalates in vegetable oils

1 Scope

This methoddocument specifies two methods for the quantitative determination of phthalates in vegetable oils by gas chromatography-mass spectrometry (GC-MS):

— Part A for the determination of di-2-ethylhexyl phthalate (DEHP) ,di-2-ethylhexyl phthalate (DEHP).

— Part B for the determination of 8eight phthalates: dimethyl phthalate (DMP), diethyl phthalate (DEP), di-isobutyl phthalate (DIBP), dibutyl phthalate (DBP), benzylbutyl phthalate (BBP), di-2-ethylhexyl phthalatedi-2-ethylhexyl phthalate (DEHP), di-isononyl phthalate (DINP) ,), di-isodecyl phthalate (DIDP).

Both methods are applicable for all vegetable oils, including crude, refined and virgin.

2 2Normative references

The following documents, are referred to in wholethe text in such a way that some or in part, are normatively referenced inall of their content constitutes requirements of this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 661, *Animal and vegetable fats and oils — Preparation of test sample* (ISO 661)

3 3Terms 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/obphttps://www.iso.org/obp

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

4 4 PARTPart A - Determination of DEHP

4.1 4.1 Principle

Phthalates are first extracted from oil with acetonitrile followed by dispersive-solid-phase extraction clean-up (Dispersivedispersive SPE), which is done to remove organic acids, excess water and other components using a combination of primary secondary amine (PSA) sorbent and C18 bonded phase. Finally, the extract is concentrated and analyzedanalysed by gas chromatography-mass spectrometry (GC-MS).

4.2 4.2 Reagents

WARNING — Attention is drawn to national regulations that specify the handling of hazardous substances, and users' obligations thereunder. Technical, organizational and personal safety measures shall be followed.

Unless otherwise specified, use only reagents of recognized analytical grade.

~~4.2.1 Unless otherwise specified, use only reagents of recognized analytical grade.~~

Toluene, purity 99 % min.

4.2.2 Acetonitrile, trace organic analysis grade, purity 99 % min.

4.2.3 n-Hexane, trace organic analysis grade, purity 99 % min.

4.2.4 Chromabond adsorbent Diamino (PSA), Chromabond adsorbent C18[1].

~~Note 1:~~ NOTE These powders can be sources of contamination. To remove the contaminants, the powders can be purified using Soxhlet extraction by extracting twice with 200 ml of n-hexane during a ~~6h~~six-hour period.

4.2.5 n-Dodecane, purity 99 % min.

4.2.6 Standards

Table 1: — CAS number of phthalates

Phthalates	~~synonym~~Synonym	~~CAS Number~~CAS Registry Number®[2]
~~di-2-ethylhexyl phthalate~~di-2-ethylhexyl phthalate	DEHP	~~117-81-7~~117-81-7
deuterated di-2-ethylhexyl phthalate	DEHP-d4	93951-87-2

4.2.6.1 Solution of DEHP-d4 internal standard, mass concentration ~~ρ=~~ρ = 50 µg/ml.

As an example, in a volumetric flask of 100 ml, weigh to the nearest mg, approximately 50 mg of DEHP-d4 and dilute to 100 ml of toluene and then proceed with a second dilution of this mixture of 10 ml ~~→~~→ 100 ml with toluene.

4.2.6.2 DEHP calibration solutions

As an example, in a volumetric flask of 100 ml, weigh to the nearest mg, approximately 50 mg of DEHP and dilute to 100 ml of toluene (SM= 500 µg/ml). Then prepare:

— 20 µl of SM and dilute to 10 ml of acetonitrile: SF1 = 1 µg/ml

— 200 µl of SM and dilute to 10 ml of acetonitrile: SF2 = 10 µg/ml

[1] Chromabond sorbent C18 ref 730611 and Chromabond sorbent Diamino (PSA) ref 730611 from Macherey Nagel (~~www.mn-net.com~~www.mn-net.com) have proved to be free of phthalate contamination. This information is given for the convenience of users of this ~~International Standard~~document and does not constitute an endorsement by ISO of these products.

[2] Chemical Abstracts Service (CAS) Registry Number® is a trademark of the American Chemical Society (ACS). This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of the product named. Equivalent products may be used if they can be shown to lead to the same results.

— 2- ml of SM and dilute to 10- ml of acetonitrile: SF3 = 100- µg/ml

Then prepare a calibration range in acetonitrile following Table 2,Table 2, using glass syringes washed 10 times with toluene and 5five times with related solutions.

Table- 2: — Example of preparation for DEHP calibration

-	[Concentration] DEHP $C_{cal\text{-}ph}$ µg/ml	Volume (µl)				[Concentration] DEHP-d4 $C_{cal\text{-}dph}$ µg/ml	Solution of DEHP-d4 internal standard (µl)	Volume of acetonitrile to be added (µl)	Total volume (µl)
		SF1	SF2	SF3	SM				
G0	**0**	-	-	-	-	**4**	80	920	10001 000
G1	**0,02**	20	-	-	-	**4**	80	900	10001 000
G2	**0,05**	50	-	-		**4**	80	870	10001 000
G3	**0,1**	-	10	-	-	**4**	80	910	10001 000
G4	**0,5**	-	50	-	-	**4**	80	870	10001 000
G5	**1**	-	-	10	-	**4**	80	910	10001 000
G6	**2**	-	-	20	-	**4**	80	900	10001 000
G7	**5**	-	-	-	10	**4**	80	910	10001 000
G8	**10**	-	-	-	20	**4**	80	900	10001 000

Note 2: Depending on the apparatus used, it is not possible to be linear from G0 to G8. In this case, it is necessary to increase the volume of acetonitrile of recovery in order to be linear over the greatest possible range while being able to see the level G1.

4.3 4.3 Apparatus

Glassware used for the determination shall be thoroughly cleaned at 550 °C during 6 hours: h, such as Pasteur pipette, vial, conical glass sample vial, 10- ml and 15- ml capacity.

4.3.1 Conical glass sample vials, 10- ml capacity.

4.3.2 Conical glass sample vials, 15- ml capacity.

4.3.3 Glass syringe, 10- µl, 20- µl, 50- µl, 100- µl, 250- µl and 500- µl capacity.

4.3.4 Automatic evaporator, for 10 ml tube (optional), recommended operating conditions: temperature of the water bath = 40 °C, nitrogen pressure = 5 psi.

4.3.5 Conical glass sample vials, 2 ml capacity.

4.3.6 Gas chromatograph, suitable for use with capillary column, equipped with an injector split-splitless or equivalent device, a temperature-programmable oven and mass detector with electron ionization source (ionization energy of 70 eV) and SIM (single ion monitoring) mode.

4.3.7 Data acquisition system, with the possibility of manual integration.

4.3.8 Capillary column, capable of being programmed up to 400 °C ("high temperature" type) for which the following characteristics are advised: 95 % dimethyl~~/~~/5 % diphenyl polysiloxane stationary phase, length of 15 m, internal diameter of 0,25 mm, film thickness of 0,1 µm or length of 30 m, internal diameter of 0,25 mm, 0,25 µm film thickness.

4.3.9 Microsyringe, 5 µl to 10 µl capacity, suitable for split less injection in gas chromatography.

4.3.10 Analytical balance, reading accuracy 0,001 g.

4.3.11 Pasteur pipette, in glass.

4.3.12 Centrifuge, capable of attaining at least 3 000 min−1, suitable for 10 ml tubes.

4.3.13 Pipettes, capable of pipetting volumes up to 10 ml.

4.4 ~~4.4~~ Sampling

A representative sample should have been sent to the laboratory. It should not have been damaged or changed during transport or storage.

Sampling is not part of the method specified in this ~~International Standard.~~document. A recommended sampling method is given in ISO 5555~~[1].~~[1]

~~Note 3: Analyze~~Analyse only sample aliquots packaged in glass bottles with suitable lids (the sample contact material from the lid should be, for example, ~~Teflon®, aluminum~~polytetrafluoroethylene (PTFE), aluminium, rubber but not plastic nor soft seal) to avoid further contamination. Plastic packaging is not suitable.

~~**Precautions:**~~

Due to the presence of phthalates in the environment, the analysis of these compounds requires precautions throughout the analysis:

— ~~-~~ ~~Avoid~~avoid contact with plastic material,

— ~~-~~ ~~Test~~test the solvents/materials used for phthalates,

— ~~-~~ A blank sample should be analysed every four samples,

— ~~-~~ ~~Glassware~~glassware that cannot be baked needs to be rinsed with a suitable solvent (acetone),

~~4/~~

