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"Fat Solubility of Solid and Liquid Substances"
(Flask Method)

1. INTRODUCTORY INFORMATION

• Prerequisite

– Suitable analytical method necessary

• Guidance information

– Partition coefficient
– Water solubility
– Structural formula
– Vapour pressure curve
– Stability at 50°C

• Coefficient of variation

The coefficient of variation on the mean values reported by the participants of the OECD Laboratory Intercomparison Testing, Part I, 1979, appeared to be dependent on the chemicals tested; it ranged from 0.035 to 0.244.

• Qualifying statements

– This test method can only be applied to pure substances.

– It is applicable only to those substances which are stable at 50°C for at least 24 hours and which also are not appreciably volatile under the same conditions. Natural fats and oils should not be used for determining fat solubility because their composition is not precisely known.

– The method is not suitable for test substances which are reactive with triglyceride.

• Recommendation

The relationships between fat solubility and partition coefficient and the bioaccumulation of a substance should be investigated.

• Standard documents

This Test Guideline is based on reference 4, Section 4, Literature.

2. METHOD

A. INTRODUCTION, PURPOSE, SCOPE, RELEVANCE, APPLICATION AND LIMITS OF TEST

The fat solubility of a substance is one of the data for evaluating the storage of lipid soluble materials in biological tissue. It is especially useful in cases in which the water solubility is too low to allow the measurement of the partition coefficient. It is also relevant to the migration of chemicals from packaging components into food stuffs.

- Definitions and units

The mass fraction of a substance which forms a homogeneous phase with a liquid fat (oil) without giving rise to chemical reactions is defined as fat solubility. The maximum of such mass fraction is called the saturation mass fraction, and this is a function of temperature.

The composition of fats will differ from organism to organism and even within a single organism. Since it is desirable to be able to compare results from different laboratories, it is necessary to employ standard fat. This standard fat should be similar in composition and behaviour to materials occurring naturally, and a commercial triglyceride mixture described in the Annex satisfies this requirement, although any other mixtures of triglycerides that give demonstrably comparable results can be used.

The saturation mass fraction of a substance should be given in g/kg of standard fat and referenced to 37°C ± 0.5°C.

The following relationship exists between the solubility in g per 1000 g of solution (S') and the solubility in g per 1000 g of solvent (S):

$$S = \frac{1000 \cdot S'}{1000 - S'}$$

- Reference substances

The reference substances need not be employed in all cases when investigating a new substance. They are provided primarily so that calibration of the method may be performed from time to time and to offer the chance to compare the results when another method is

applied. The values presented below are not necessarily representative of theresults which can be obtained with this test method as they have been derived from an earlier version of the Test Guideline.

	Fat solubility* (in g/1000 g (fat))
hexachlorobenzene	11.4 (11.1 - 12.1) (OECD)
mercury (II) chloride	20.1 (14.7 - 24.3) (OECD)
urea	0.17 (0.05 - 0.28) (EEC)

- Principle of the test method

The substance is dissolved in a liquid "standard fat" by stirring, and the saturation mass fraction of the substance is achieved by continued addition until a constant value for the mass fraction dependent variable is achieved as determined by a suitable analytical method.

- Quality criteria

Repeatability

The repeatability of the measurement is unknown at present. It will develop from the analytical procedure which in turn is related to the substance. It should be reported as a standard deviation about the mean as indicated elsewhere in this Test Guideline.

Sensitivity

The sensitivity of the method is determined by the sensitivity of the analytical process.

Specificity

Results should apply to the "standard fat" and are appropriate for relatively pure substances. Even at 37°C, the standard fat may form emulsions or fine suspension of solid substances. Since these will interfere with subsequent determination of mass fraction, they must be avoided.

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* Total mean and range of mean values (in brackets reported by the participants of the OECD or EC Laboratory Intercomparison Testing programme.

B. DESCRIPTION OF TEST PROCEDURE

- Preparations

Apparatus

The following items of equipment are required:

– normal laboratory glassware
– balance
– centrifuge with thermostat
– a stirrer which can be used in combination with a temperature control system
– thermostat.

Preliminary test

A simplified preliminary test should be run to determine the approximate amount of substance necessary for the establishment of the saturation mass fraction at the test temperature (37°C).

Note: The rate of establishment of the saturation equilibrium may be greatly dependent upon the particle size in the case of solid substances. For this reason, such materials should be pulverised.

Preparation of the substances

Weigh eight samples into 50 ml flasks. Each should be twice the quantity necessary for saturation as determined in the preliminary test.

After adding a weighed amount of approximately 25 g of liquified and mixed standard fat, the flasks fitted with the stirrers are tightly closed with ground-glass stoppers. One half (group I) is stirred at 30°C, and the other half (group II) at approximately 50°C, each for at least one hour.

- Test conditions

The determination of fat solubility is carried out at 37°C ± 0.5°C.

- Performance of the test

Stir the contents of the flasks in both groups at 37°C ± 0.5°C.

The stirring time required to establish equilibrium cannot be predicted in general. In the case of liquid substances, saturation may be reached within minutes; in the case of solid substances it may take hours. For liquids, three hours of stirring should be sufficient, after which stirring should be stopped for two of the flasks in both groups and these two flasks allowed to stand for at least one hour at 37°C in order to separate the undissolved amount of substance and the formation of a homogeneous phase. In the event of emulsion or suspension formation (e.g. Tyndall effect), this must be eliminated by a suitable method such as thermostated centrifugation.

The third and the fourth flask in both groups should be stirred for at least 24 hours before standing for one hour at 37°C ± 0.5°C.

Note: If no bottom sediment (for solid substances) or no phase separation (for liquid substances) has formed after this period, the test must be repeated with a greater amount of substance.

- Analysis

One sample is taken from each saturated fat phase for analysis. This sample is weighed, and the mass fraction is determined using a substance-specific analytical method.

Any suitable method may be used for this, e.g.

– photometric methods
– gas chromatography
– extraction with water and subsequent determination either directly in this medium or after back extraction to an organic solvent.

3. DATA AND REPORTING

- Treatment of results

If there are significant differences in results from either under or oversaturation or short and long time periods, the test should be repeated with longer stirring times.

• Test report

The results should be evaluated as described above and they are part of the test report. If there were no significant differences between the various observed values in gram per kilogram, the individual values, the mean value and the standard deviation should be reported. If there are significant differences, even after retesting, then only the individual results should be reported.

The following items should also be included in the report of fat solubility:

– substance (any detail about preparation, identities, etc.)

– fat (e.g. description, characteristics, origin)

– method of analysis, deviations and special features.

4. LITERATURE

1. H. Rheinboldt in Houben: *Die Methoden der organischen Chemie*, Vol. 1, p. 866 (1925).

2. H. Kienitz in Houben-Weyl: *Methoden der organischen Chemie* 3/1, p. 219 (1955).

3. W. J. Mader, R. D. Vold & M. J. Vold in J. Weissberger: *Technique of Organic Chemistry* I/I, p. 655 (1966).

4. ASTM D 2780, Test for solubility of fixed gases in liquids.

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5. A N N E X

STANDARD FAT

A standard fat which is available commercially from the NATEC Company, Gesellschaft für naturwissen-schaftlich-technische Dienste mbH, Behringstrasse 154, D-2000 Hamburg 50, is HB 307. This fat simulant is a synthetic mixture of saturated triglycerides with a fatty acid and triglyceride distribution similar to that of a coconut fat.

The following table shows the composition of a typical batch of HB 307:

Fatty acid distribution

Number of C-atoms in the fatty acid moiety	6	8	10	12	14	16	18	others
GLC areas (%)	0.5	7.5	10.3	50.4	13.9	7.8	8.6	1

Glyceride distribution

Total number of C-atoms in the fatty acid moieties	22	24	26	28	30	32	34	36	38
GLC areas (%)	0.1	0.3	1.0	2.3	4.9	10.9	13.9	21.1	16.1

	40	42	44	46	48	50
	11.7	9.8	4.4	2.2	1.1	0.2

Purity

Monoglyceride content (enzymatic)	≤ 0.1%
Diglyceride content (enzymatic)	≤ 0.4%
Unsaponifiable content	≤ 0.1%
Wijs number	≤ 0.5%
Acid number	0.02%
Water content (K. Fischer)	≤ 0.1%
Clear melting point	28.5°C

Typical Absorption Spectrum (layer thickness d = 1 cm, comparison: water, 35°C)

Wavelength (nm)	290	310	330	350	370	390	430	470	510
Transmission (%)	2	15	37	64	80	88	95	97	98

At least 10 per cent light transmission at 303 nm.