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**Animal and vegetable fats and oils —  
Determination of tocopherol and  
tocotrienol contents by high-performance  
liquid chromatography**

**AMENDMENT 1: Updating of reagents and  
confirmation of statistical data validity**

*Corps gras d'origines animale et végétale — Détermination des teneurs  
en tocophérols et en tocotriénols par chromatographie en phase liquide  
à haute performance*

*AMENDEMENT 1: Mise à jour des réactifs et confirmation de la validité  
des données statistiques*



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## Foreword

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Amendment 1 to ISO 9936:2006 was prepared by Technical Committee ISO/TC 34, *Food products*, Subcommittee SC 11, *Animal and vegetable fats and oils*.

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# Animal and vegetable fats and oils — Determination of tocopherol and tocotrienol contents by high-performance liquid chromatography

## AMENDMENT 1: Updating of reagents and confirmation of statistical data validity

Page 2, 5.1

Replace the Note with the following text and associated footnotes:

$\alpha$ -,  $\beta$ -,  $\gamma$ - And  $\delta$ -tocopherol standards can be obtained from Merck<sup>1</sup>);  $\alpha$ -tocopherol can be obtained from various suppliers. Tocotrienol standards are available from Sigma Aldrich<sup>2</sup>). It has been reported that the purity of some commercially available tocopherol standards can vary between 85 % and 100 %. Thus, it is important to determine the concentration of prepared calibration solutions by UV spectrometry (see 9.1.1).

Page 2, 5.4

Replace the entire subclause with the following text:

**5.4 HPLC mobile phase:** any suitable mixture of solvents that has been proved to reach a chromatographic resolution of peaks as good as the one presented in Table 2 (relative retention time of tocopherols and tocotrienols) and in Annex A (chromatograms of a mixture of vegetable oils) should be used (see Table C.3).

A good separation of  $\gamma$ -tocopherol and  $\beta$ -tocotrienol can be achieved by using a mixture of 5 % volume fraction *t*-butyl methyl ether + 95 % volume fraction *n*-heptane and a diol-column.

The preparation of a suitable mobile phase, 3,85 % volume fraction tetrahydrofuran solution in *n*-heptane, for use with silica columns is as follows. Using a 1 000 ml graduated cylinder (6.5), introduce 1 000 ml of *n*-heptane (5.3) in a 2 l bottle. Add two 20 ml volumes of tetrahydrofuran (5.2) using a 20 ml volumetric pipette (6.6). Homogenize the mobile phase by means of an ultrasonic bath (6.8) for 15 min.

Page 3, 6.2

In Note 1, renumber as 3) the footnote and the reference to it.

Add the following note:

NOTE 3 Both types of columns have been used for the evaluation of the precision data (Annex C).

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1) Merck Tocopherol set 613424 is available from Calbiochem ([www.calbiochem.com](http://www.calbiochem.com)). It contains one 50 mg vial each of DL- $\alpha$ -tocopherol, D- $\beta$ -tocopherol, D- $\gamma$ -tocopherol, and D- $\delta$ -tocopherol with a purity of 95 % by HPLC (for each component). This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of this product.

2) Tocotrienols are available from Sigma Aldrich ([www.sigmaaldrich.com](http://www.sigmaaldrich.com)) and from Chromadex ([www.chromadex.com](http://www.chromadex.com)) with purities between 65 % and 98 %. This information is given for the convenience of users of this International Standard and does not constitute an endorsement by ISO of these products.

Page 3, 6.5, 6.6, 6.7

Update the text to read:

- 6.5 Graduated measuring cylinder**, capacity 1 000 ml, ISO 4788<sup>[7]</sup>, class A.
- 6.6 Volumetric pipettes**, capacities 10 ml and 20 ml, ISO 648<sup>[5]</sup>, class A.
- 6.7 One-mark volumetric flasks**, capacities 50 ml and 25 ml, ISO 1042<sup>[6]</sup>, class A.

Page 12, Annex C

Replace the second paragraph with:

The various types of HPLC mobile phases used by the participants in this test are listed in Table C.3. While six participants used a silica gel column, six participants also used a diol column. Therefore, the statistical results, given in Table C.2, are valid for both types of columns.

Page 17, Bibliography

Add the following entries:

- [5] ISO 648, *Laboratory glassware — Single-volume pipettes*
- [6] ISO 1042, *Laboratory glassware — One-mark volumetric flasks*
- [7] ISO 4788, *Laboratory glassware — Graduated measuring cylinders*