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Method ring test
MOSH/MOAH in fish oil
(LC-GC-FID and GC×GC-FID)
P2605-MRT



Summary

The entire report is available to participants only.

The method ring test is designed, realised, evaluated, and authorised on behalf of PROOF-ACS GmbH by

Dr. Birgit Schindler
Managing Director PROOF-ACS GmbH
Project coordinator

The report is approved by



Dr. Birgit Schindler
cn=Dr. Birgit Schindler,
o=PROOF-ACS GmbH,
ou=Managing Director,
email=proof@proof-acs.de,
c=DE
2026.10.26 10:13:25 +01'00'

Dr. Birgit Schindler

Participants with any comments or concerns related to this ring test are invited to contact:

PROOF-ACS GmbH
Gottlieb-Daimler-Str. 1
28237 Bremen
Phone: +49 421 388 928 50
E-mail: proof@proof-acs.de
www.proof-acs.de

DAkkS
Deutsche
Akkreditierungsstelle
D-EP-22211-01-00

PROOF-ACS is a DAkkS accredited proficiency testing provider according to DIN EN ISO 17043:2023 (D-EP-22211-01-00). This method ring test is covered by the scope of accreditation.

PROOF-ACS GmbH does not have any analytical laboratory facilities of its own. Homogeneity testing and stability testing are subcontracted to laboratories, accredited according to DIN EN ISO 17025. The subcontracted laboratory may also participate in the ring tests. If so, the laboratory is treated in the same way as other participants and the same rules of confidentiality apply.

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Method ring tests are a highly valuable instruments to gather deep insight into the real challenges of complex analytical methods like the quantification of MOSH and MOAH. Two different techniques, LC-GC-FID and GC×GC-FID are applied for quantification in routine analysis. This method ring test offers the chance to apply and compare both techniques to the same samples.

The method ring test consists of three parts:

- Part 1: Evaluation of the analytical results

  The performance of laboratories is evaluated with respect to their ability to quantify MOSH and MOAH in fish oil (EPA/DHA omega-3 oil).

- Part 2: The applied analytical methods

  Details related to the applied analytical methods are summarised and considered for interpretation of the analytical results.

- Part 3: Chromatograms

  The analytical procedure in quantifying MOSH and MOAH is based on the integration of the respective “humps”. The chromatograms of all laboratories are collected and summarised. Conspicuous chromatograms are discussed in the report and are considered for the interpretation of the analytical results. The plots related to the determination by GC×GC-FID are collected and summarised as well.

Fish oil is one of the highly challenging matrices in MOSH/MOAH analysis. Depending on the quality of the fish oil, a high level of interferences might complicate the sample clean-up and the integration of the humps. For that reason, two different types of fish oil are provided. A semi-refined fish oil (EPA/DHA omega-3 oil) is spiked with MOSH and MOAH and provided as test material. The corresponding unspiked fish oil is provided as blank material. The evaluation of the performance of the labs is based on the analytical result related to the test material. Another material, a crude fish oil, is provided as sample for training purposes only. The material is expected to be more challenging due to more interferences. It offers laboratories the chance to gather and exchange knowledge for highly challenging fish oil matrices.

10 laboratories across five countries (China, Chile, France, Germany, and Netherlands) took part in the test. 9 labs reported results and are considered for evaluation.

The laboratories are asked to report analytical results related the test material, the blank material, and the sample for training purposes. The labs are free to choose both techniques, LC-GC-FID and GC×GC-FID or just the more common LC-GC-FID.

Besides the pure analytical data, the laboratories are asked to provide comprehensive data related to the applied analytical methods in a questionnaire and chromatograms and plots related to the test material, the blank material, the sample for training purposes, and related to reagent blank samples.

Analytical results are reported related to

- MOSH ≥ n-C10 to ≤ n-C16 (LC-GC-FID)
- MOSH > n-C16 to ≤ n-C20 (LC-GC-FID)
- MOSH > n-C20 to ≤ n-C25 (LC-GC-FID)
- MOSH > n-C25 to ≤ n-C35 (LC-GC-FID)
- MOSH > n-C35 to ≤ n-C40 (LC-GC-FID)
- MOSH > n-C40 to ≤ n-C50 (LC-GC-FID)
- Total MOSH (LC-GC-FID)
- MOAH ≥ n-C10 to ≤ n-C16 (LC-GC-FID)
- MOAH > n-C16 to ≤ n-C25 (LC-GC-FID)
- MOAH > n-C25 to ≤ n-C35 (LC-GC-FID)
- MOAH > n-C35 to ≤ n-C50 (LC-GC-FID)
- Total MOAH (LC-GC-FID)
- Total MOAH (GC×GC-FID)

The labs are asked to report the results related to LC-GC-FID in accordance with the "Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials – 2nd Edition", published by the Joint Research Centre of the European Commission.

According to the guidance document, total MOSH and total MOAH should be determined as follows:

> *„The "total MOSH/MOAH content" (n-C10-C50) is determined by integrating the chromatogram,*
> *- from the retention time of the beginning of the n-C10 peak;*
> *- to the retention time of the end of the n-C50 peak;*
> *- after the trimming of the riding peaks […] above the hump(s); and*
> *- after the subtraction of/adjustment for the reagent blank (baseline).*
>
> *The obtained "corrected hump" should be an unambiguously identified smooth hump" (page 15).*

The results related to total MOSH (LC-GC-FID) and total MOAH (LC-GC-FID and GC×GC-FID) are considered for evaluation. The results related to the individual fractions of MOSH and MOAH (LC-GC-FID) are summarised for information only.

The evaluation of the results related to the blank material and related to the sample for training purposes is provided for information only. The results related to the test material are considered to evaluate the performance of the laboratories in the test.

The raw material (= blank material) contains a contamination with 7.00 mg/kg of MOSH (assigned value) and 1.74 mg/kg of MOAH (assigned value).

The performance of laboratories in the test is evaluated according to

- the *comparability* of the results. The evaluation of the comparability is based on the z-score model. The absolute values of z-score should be at least ≤ 2. The comparability criterion is applied to total MOSH and total MOAH related the analysis by LC-GC-FID. It is not applicable to the results related to the analysis by GC×GC-FID due to the limited number of reported results.
- the *trueness* of the results. The trueness is expressed as the coverage of the spiked level in %. The coverage should be at least between 70 and 120 % of the spiked level. The trueness criterion is applied to total MOSH by LC-GC-FID, and to MOAH by LC-GC-FID and GC×GC-FID. The contamination of the blank material with MOSH and MOAH is considered for evaluation of the trueness of results related to the test material.

The statistical evaluation of the results is summarised in the tables below:

Matrix	Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Total number of results	**Comparability:** no. of results, which correspond to |z-score| ≤ 2	**Trueness:** no. of results, which correspond to recoveries of 70 to 120 % of the spiked level
Blank material	Total MOSH	unspiked	7.00	9	Not evaluated	Not evaluated
	Total MOAH (LC-GC-FID)	unspiked	1.74	9	Not evaluated	Not evaluated
	Total MOAH (GC×GC-FID)	unspiked	-	5	Not evaluated	Not evaluated
Test material	Total MOSH	20	27.1*	9	8	8
	Total MOAH (LC-GC-FID)	9.8	10.2*	9	9	9
	Total MOAH (GC×GC-FID)	9.8	-	5	Not applicable	4

* The assigned value corresponds to the level of MOSH/MOAH in the blank material plus the spiked level.

The standardised analytical method DIN EN ISO 20122:2024 or similar is applied by most of the labs. Effects of increasing standardisation and harmonisation are seen over the last years. Even though the standardised method is now available, the analytical methods still differ in detail. Different approaches and concepts for clean-up are applied by the laboratories. Aluminium oxide, epoxidation, saponification, and/or silica gel are chosen for

clean-up depending in the preferences of the labs. However, knowledge and expertise are still needed to avoid false positive and false negative findings as well as incorrect interpretation of the results. Standardisation is still needed with respect to the conditions of epoxidation.

A reverse setup (mid-polar × non-polar) or a normal setup (non-polar × mid-polar) is applied for quantification by GC×GC-FID.

The details related to the applied analytical methods are summarised in part 2 of the report. Part 3 of the report summarises the chromatograms and plots of all participants. Parts 2 and 3 of the report are dedicated to the analytical specialists in the labs. Combining the analytical details to the resulting chromatograms can support the laboratories to improve the quality of the applied analytical methods and to learn from each other.

The results of the labs show that for fish oil samples like the matrix that is used for the test material and the blank material, a quantification at the planned maximum level of MOAH of 5 mg/kg is well feasible for the labs. For other types of fish oil might be more challenging.