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Method ring test
MOSH/MOAH in edible oils (LC-GC-FID)
P2301-MRT

Summary

The entire report is available to participants only.

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

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The report was approved by

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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.

Method ring tests are a highly valuable instruments to gather deep insight into the real challenges of complex analytical methods like the quantification of low levels of MOSH and MOAH in complex matrices like edible oils.

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 two different samples of edible oils.

- 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.

Coconut oil and sunflower oil are chosen as matrices for the method ring test. An unspiked sample as well as a spiked sample of each oil are provided as blank materials resp. test materials. The oils are spiked with a technical white oil, a grease oil, and lubricant oils.

20 laboratories across six countries (France, Germany, Italy, Malaysia, Netherlands, and Spain) took part in the test. 19 laboratories reported results and are considered for evaluation. 17 laboratories analysed the samples related to the matrix coconut oil, while 19 labs analysed the samples related to the matrix sunflower oil.

The laboratories were asked to report analytical results related the test material and the blank material. Besides the pure analytical data, the laboratories were asked to provide comprehensive data related to the applied analytical methods in a questionnaire and chromatograms related to the test materials and the blank materials and related to reagent blank samples.

Analytical results were reported related to the fractions:

- MOSH ≥ n-C10 to ≤ n-C16
- MOSH > n-C16 to ≤ n-C20
- MOSH > n-C20 to ≤ n-C25
- MOSH > n-C25 to ≤ n-C35
- MOSH > n-C35 to ≤ n-C40
- MOSH > n-C40 to ≤ n-C50
- Total MOSH

- MOAH ≥ n-C10 to ≤ n-C16
- MOAH > n-C16 to ≤ n-C25
- MOAH > n-C25 to ≤ n-C35
- MOAH > n-C35 to ≤ n-C50
- Total MOAH

in accordance with the Guidance of the Joint Research Centre of the EU.

According to the guidance document of JRC (1), total MOSH and total MOAH should be determined as follows:

> *„The parameters "total MOSH/MOAH" should be determined by integration of the whole signal interval in the chromatogram, starting at the retention time of the peak start of n-C10 and ending at the retention time of the peak end of n-C50 after the elimination of the identified sharp peaks above the hump and if possible, elimination of POH and/or POA signals.“ (page 16).*

Total MOSH and total MOAH determined in accordance with the guidance document of JRC are considered for evaluation in this method ring test. The results are marked as (total hump) in the report.

The evaluation related to the lower bound results is provided in the annex of part 1 of the report for information only. The lower bound approach means, results < LOQ are considered as “0” during the calculation of the sum of the different fractions. Especially in case of low levels of MOSH and MOAH the lower bound approach results in an underestimation of the true levels of MOSH and MOAH.

Both blank materials are free from MOAH (< 1 mg/kg). The blank material coconut oil contains trace levels of total MOSH of about 1 mg/kg, while the blank material sunflower oil contains total MOSH at a level about 2 mg/kg. The levels of MOSH in the blank materials are considered for evaluation of the test materials.

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 z-score should be at least ≤ |2|. The comparability criterion is applied to total MOSH and total MOAH related to both oils. The evaluation of the individual fractions of MOSH and MOAH is provided for information purposes only.
- 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 and total MOAH related to both oils. The levels of total MOSH in the blank materials are considered during evaluation of the test materials.

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

Blank material

Matrix	Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Total number of results
Coconut oil	Total MOSH (total hump)	unspiked	1.07	11
	Total MOAH (total hump)	unspiked	< 1.00	17
Sunflower oil	Total MOSH (total hump)	unspiked	2.11	19
	Total MOAH (total hump)	unspiked	< 1.00	19

Test material

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
Coconut oil	Total MOSH (total hump)	7.1	7.51	17	13	14
	Total MOAH (total hump)	4.8	3.16	17	14	6
Sunflower oil	Total MOSH (total hump)	3.3	4.83	19	13	11
	Total MOAH (total hump)	3.2	2.15	19	13	9

Several approaches took place to harmonise the analytical methods, which are applied for quantification of MOSH and MOAH in oils throughout the last years.

Analytical methods were improved to fit to even low levels of MOSH and MOAH of about 1 mg/kg in edible oils. Clean-up procedures and especially the conditions for epoxidation were improved.

However, still 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. Depending on the level of knowledge and the level of experience, the outcome might differ a lot. The different types of clean-up might have a large impact on the validity of the resulting data. If the same type of clean-up is applied, the procedures might be rather different in detail.

If clean-up procedures like aluminium oxide and epoxidation are not applied appropriately, the respective labs overestimate the content of MOSH and MOAH due to misinterpretation of interferences.

Compared to the first MOSH/MOAH method ring tests of PROOF (P1917-MRT, P1918-MRT, P2016-MRT), the performances of the laboratories improved a lot. However, further laboratories established the analysis of MOSH and MOAH in the labs over the last year. The laboratories are less experienced and the performance with respect to the sample preparation and the chromatography is not at the same high level. Typical issues related to chromatographic issues are discussed in section 6 of part 1 of the report. The chromatograms of all labs are summarised in part 3 of the report.

The overall performance of the labs is quite satisfying with respect to the quantification of total MOSH. The matrix coconut seems to be easier than the matrix sunflower oil. The spiked level is higher for coconut oil (7.1 mg/kg) compared to the spiked level related to sunflower oil (3.3 mg/kg). An alox clean-up helps to reduce interferences for the matrix sunflower.

In general, the quantification of total MOAH is more challenging than the quantification of MOSH. The spiked levels of 4.8 mg/kg (coconut oi) resp. 3.2 mg/kg (sunflower oil) are comparably low and close to the LOQs of most of the labs of 1 mg/kg. The labs tend to underestimate the level of MOAH in both test materials. The assigned values correspond to 66 % (coconut oil) resp. 67 % (sunflower oil) of the spiked levels. However, if an adequate sample preparation is applied and chromatographic issues are solved, recoveries of up to 100 % of the spiked levels are feasible.

In common proficiency tests, the statistical evaluation is limited to the comparability of the results. However, the comparability is just a first step, especially in case of challenging analytical methods. Much deeper insights are possible if the trueness criterion is applied, and if the information related to the applied analytical methods is combined with the provided chromatograms for evaluation.
The summary of the applied analytical methods (part 2 of the report) can support laboratories to improve the quality of the applied analytical method e.g. the choice of the most suitable conditions for epoxidation. Furthermore, the method details can build the basis for further discussion and thus for a standardisation of the analytical methods related to MOSH and MOAH.

The submitted chromatograms of all participants are summarised in part 3 of the report. The provided chromatograms allow for a deep insight in the challenges of quantifying MOSH and MOAH. The chromatograms thus offer a chance to each laboratory to compare the own outcome of the analytical methods to those of other laboratories on the market. Is the chromatography in line with the state-of-the-art or does it need an improvement?

Some of the major challenges by means of the analytical methods and chromatography to be solved are:

- The choice of a suitable method for clean-up (e.g. aluminium oxide and epoxidation).
- An adequate application of the clean-up and thus a satisfying removal of interfering substances.
- A sufficient sensitivity (e.g. by sufficient pre-concentration).
- An adequate identification and interpretation of interferences.

Analysing MOSH and MOAH is not plug-and-play and requires a high level of experience, especially if low levels of MOSH and MOAH are quantified. Major parts of the analytical procedure are highly automated, however an adequate clean-up as well as suitable chromatographic conditions are necessary for a reliable quantification. Expert knowledge is indispensable for a correct interpretation of the resulting chromatograms. The laboratories must be able to identify interferences to avoid misinterpretation and thus overestimation of the true values of MOSH and MOAH.

If the labs are experienced and sophisticated analytical methods are correctly applied, a reliable, comparable, and true quantification of MOSH and MOAH in edible oils is possible, even at low levels.