Facing analytical quality.

Method ring test
MOSH/MOAH
in sunflower oil and rapeseed oil
P2016-MRT

Summary

The entire report is available to participants only.

Designed, realised and evaluated by

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May 2020,

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Method ring tests like P2016-MRT are a highly valuable instruments to gather deep insight into the real challenges of complex analytical methods like the quantification of MOSH and MOAH in foodstuff.

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 oil (sunflower oil/rapeseed 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.

Two different samples of edible oil were provided as test materials:

  - sunflower oil, spiked with Shell Gadus and a technical creeping oil, and
  - rapeseed oil, spiked with Shell Gadus and a food grade lubricant.

Blank materials of the corresponding sunflower oil resp. rapeseed oil were provided upon request.

13 laboratories across four countries (France, Germany, Italy, and Netherlands) took part in the test. 12 of the labs reported results and are considered for evaluation. The laboratories were asked to report analytical results related to both test materials and related to both blank materials (if ordered). 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 all test and 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.

In routine, total MOSH and total MOAH are usually calculated of the results related to the different fractions according to the lower bound approach. The lower bound approach means, results < LOQ are considered as "0" during the calculation of the sum of the different fractions.

According to the guidance document of JRC (5), 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).*

The approach described by JRC is thus different from the lower bound approach. In this method ring test, the laboratories were asked to report the results related to total MOSH and total MOAH as

a) lower bound of total MOSH/total MOAH, and
b) total hump of total MOSH/total MOAH (according to JRC).

The results related to the total hump of total MOSH and total MOAH are considered for evaluation in this method ring test. The lower bound results of total MOSH and total MOAH are provided for information only.

Both blank materials contain (low) levels of total MOSH of about 1 mg/kg (sunflower oil) resp. 2.5 mg/kg (rapeseed oil), while total MOAH in the blank materials is < 1 mg/kg (sunflower oil) and 1.1 mg/kg (rapeseed oil). The levels of MOSH in the blank material are not considered for the calculation of the recoveries of the spiked levels (trueness criterion), since the levels are negligible compared to the spiked levels. However, the levels of MOSH and MOAH are considered if laboratories report results slightly above the accepted range with respect to the trueness criterion.

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. The evaluation of the individual fractions of MOSH and MOAH is provided for information purposes.
- 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.

The statistical evaluation of the results is summarised in the table 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
Sunflower oil	Total MOSH	24.5	23.4	12	9	11
	Total MOAH	2.9	2.84	11	7	8
Rapeseed oil	Total MOSH	40.8	39.7	12	7	10
	Total MOAH	5.9	6.04	12	8	9

Up to now, no standardised analytical method is available for the quantification of MOSH and MOAH in edible oils at concentration levels of 1-5 mg/kg. Consequently, different approaches and concepts for clean-up are applied by the participants in this method ring test. Aluminium oxide, epoxidation, saponification, or silica gel and silver nitrate are chosen for clean-up depending in the preferences of the labs. The different types of clean-up might have a large impact on the validity of the resulting data. And even within the same type of clean-up the procedures might be rather different.

One of the main issues of standardisation is the use of a common standard for the correction of MOAH. At the moment 1-MN, 2-MN or TBB are used, which is one of the major sources for differing results of MOAH.

If clean-up procedures like aluminium oxide and epoxidation are not applied correctly, the respective labs overestimate the content of MOSH and MOAH in oil samples. This effect was observed for the reported levels of MOSH and MOAH related to the two blank materials.

Compared to the last method ring tests of PROOF (P1917-MRT, P1918-MRT), the performances of the laboratories improved a lot. Most of the laboratories are now able to perform a suitable clean-up by means of aluminium oxide, saponification and epoxidation. The chromatograms are much better, e.g. the resolution of the hump from the solvent peak is a challenge, which is overcome by most of the labs. The sensitivity of the applied methods increased and more than 50 % of the participants are now able to provide reliable results even at lower concentration levels of MOAH.

Four labs provided comparable and true results related to total MOSH and total MOAH in both edible oils. The assigned values of total MOSH and total MOAH are close to the spiked levels for both parameters and both matrices (95 to 102 % recovery of the spiked levels).

In common proficiency tests, the statistical evaluation is limited to the comparability of the results. However, the comparability is just the first step, especially in case of challenging analytical methods. Much deeper insights are possible if the trueness criterion is applied, and the information in the questionnaire is combined with the analytical data and the provided chromatograms.

The 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 remain still unsolved for some of the labs:

- 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.

The quantification of MOSH and MOAH remains challenging even for matrices like pure oils, which are supposed to be easy compared to complex foodstuffs. 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.