Facing analytical quality.

Method ring test
MOSH/MOAH
in palm oil
P1917-MRT

Summary

The entire report is available to participants only.

Designed, realised and evaluated by

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September - October 2019,

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Method ring tests like P1917-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 palm 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 palm oil were provided as test materials:

  - test material 1, spiked with Shell Gadus and a technical creeping oil, and
  - test material 2, spiked with a white oil, containing MOAH only.

Unspiked samples of the two palm oils were provided to the participants upon request.

16 laboratories across five countries (Germany, Italy, Malaysia, Netherlands and Switzerland) took part in the test. 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 are 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.

Both unspiked palm oil samples (raw materials) contain (low) levels of MOSH and MOAH. Blank material palm oil 1 contains about 2 mg/kg total MOSH and ≤ 1 mg/kg total MOAH. Blank material palm oil 2 contains about 14 mg/kg total MOSH and < 3 mg/kg total MOAH.

False positive findings were reported of

- total MOAH in blank material palm oil 1 (6.9 mg/kg and 21.3 mg/kg),
- total MOAH in blank material palm oil 2 (9 mg/kg and 21.4 mg/kg).

The reasons for the misinterpretation of levels of MOAH in the samples might be an inadequate epoxidation, a lack of experience and thus a misinterpretation of the interferences.

The results related total MOSH and total MOAH in both test materials (palm oil 1 and palm oil 2) are evaluated with respect to the comparability criterion (z-score model). Results, which correspond to z-scores ≤ |2| are considered satisfying.

The statistical evaluation is focussed on the results related to total MOSH and total MOAH (see table below). The results related to the individual fractions are provided for information purposes.

Summary of the statistical evaluation:

Matrix	Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Total number of results	**Comparability:** no. of results, which correspond to z-score ≤ |2|
Palm oil 1	Total MOSH	*31.5*	32.6	16	11
	Total MOAH	*3.50*	4.11	16	6
Palm oil 2	Total MOSH	-	14.2	16	9
	Total MOAH	*5.60*	6.19	16	6

* The spiked levels are provided for information only due to levels of MOSH and MOAH in the raw material.

Up to now, no standardised analytical methods are available for the quantification of MOSH and MOAH in food. Different approaches and concepts for clean-up are applied. Aluminium oxide, epoxidation, saponification, or silica gel and silver nitrate are chosen for clean-up depending in the preferences of the labs and depending on the matrices. And even if the same type of clean-up is chosen, the procedures might be rather different.

As a consequence, there was a need for a proficiency test, covering difficult matrices like palm oils and realistic concentration levels of MOSH and MOAH typical for the matrix and close to the LOQs of the laboratories.

With respect to total MOSH the comparability is quite good. 11 out of 16 laboratories (69 %) pass the comparability criterion related to palm oil 1 and 9 out of 16 laboratories (56 %) pass the comparability criterion related to palm oil 2. The repeatability of the analysis of MOSH is also good. Since test material palm oil 2 was spiked for MOAH only the blank material and the test material contain identical concentration levels of MOSH. The laboratories showed a

high repeatability of the MOSH results, since they were able to report comparable results for the blank material and the test material palm oil 2. The assigned values related to blank material palm oil 2 and test material palm oil 2 (13.9 resp. 14.2 mg/kg) are similar as well.

The quantification of MOAH is more challenging especially in case of low levels like in this ring test. Spiked levels were 3.5 mg/kg in test material palm oil 1 and 5.6 mg/kg in test material palm oil 2. Three laboratories reported significantly too high results related to MOAH in both test materials due to misinterpretation of interferences. The provided chromatograms reveal insufficient epoxidation of the samples for two of the labs. The same labs reported false positive results resp. comparably high levels of MOAH in the blank materials.

The overall comparability of the results related to total MOAH is low. 6 out of 16 labs pass the comparability criterion for each of the two test materials. Comparability of the results related to MOAH can be increased by a standardisation of the epoxidation as well as by agreeing upon a convention with respect to the correction of MOAH with 1-MN, 2-MN or TBB. LOQs of 0.5 mg/kg for each fraction of MOSH and MOAH can only be reached if sample clean-up is improved and samples are concentrated before analysis.

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.

The trueness of the results is an important criterion for evaluation, especially for complex analytical methods like the quantification of MOSH and MOAH. The trueness criterion cannot be applied directly due to levels of MOSH and MOAH in both raw materials. However, for information purposes, the reported concentration levels of total MOSH and total MOAH were evaluated with respect to the spiked level (trueness), considering the levels of total MOSH and total MOAH in the raw material. For that purpose, PROOF calculated the difference between the results reported related to the test material and the results reported related to the blank material. The evaluation with respect to the trueness is provided to the participants as an additional information but it is not considered for the evaluation of the performance of the laboratories in the test.

A pure statistical evaluation of the analytical data is insufficient to improve the applied analytical methods. Details related to the applied analytical methods are thus provided to the participants as a comprehensive summary in part 2 of the report. The summary can support the laboratories to identify individual shortcomings, improve the quality of the applied analytical method e.g. by means of the choice of the type of clean-up and an improvement of the methodological details of the clean-up procedure of e.g. the 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.

All submitted chromatograms are provided to the participants of the method ring test 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?

The major challenges by means of the analytical methods and chromatography were:

- 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.
- a satisfying chromatographic separation of the solvent peak. Tailing of the solvent peak might, besides others, be a result of separation columns with insufficient quality, which were available on the market recently.
- a straight baseline especially at the end of the run to avoid humps in a scrape.
- 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 especially MOAH.

Most of the laboratories are be able to present the resulting chromatograms in a proper way now. The laboratories are able to demonstrate which peaks are subtracted and which part of the hump is used during integration.