

Method ring test MOSH/MOAH in cocoa butter and chocolate bar P2502-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

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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.

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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.

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 matrices, chocolate bar and cocoa butter.
- 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.

Commercially available chocolate bars as well as cocoa butter are chosen as matrices for the method ring test. Both samples are provided unspiked (blank material) as well as spiked with MOSH and MOAH. Both unspiked materials contain low levels of MOSH, while both materials are free from MOAH.

15 laboratories across five countries (Germany, Greece, India, Italy, and Netherlands) took part in the test. All labs reported results and are considered for evaluation.

The laboratories are asked to report analytical results related the test materials and the blank materials. Besides the pure analytical data, the laboratories are 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 \geq n-C10 to \leq n-C16
- MOSH $>$ n-C16 to \leq n-C20
- MOSH $>$ n-C20 to \leq n-C25
- MOSH $>$ n-C25 to \leq n-C35
- MOSH $>$ n-C35 to \leq n-C40
- MOSH $>$ n-C40 to \leq n-C50
- Total MOSH
- MOAH \geq n-C10 to \leq n-C16
- MOAH $>$ n-C16 to \leq n-C25
- MOAH $>$ n-C25 to \leq n-C35
- MOAH $>$ n-C35 to \leq n-C50
- Total MOAH

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 and total MOAH are considered for evaluation. The results related to the individual fractions of MOSH and MOAH are summarised for information only.

The results related to total MOSH in the blank materials are evaluated with respect to the comparability criterion for information only.

The raw material (= blank material) chocolate bar contains 2.56 mg/kg total MOSH (assigned value), while the blank material cocoa butter contains 6.08 mg/kg total MOSH (assigned value). The blank material chocolate bar is free from MOAH (< 0.5 mg/kg), while the blank material cocoa butter contains trace levels of MOAH of 1 mg/kg or lower. The levels of MOSH in the blank materials are considered for evaluation of the results related to the test materials with respect to the trueness criterion. The levels of MOAH are negligible.

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 to both matrices.
- 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 matrices. The levels of MOSH in the blank material are considered for evaluation.

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
Chocolate bar	Total MOSH	unspiked	2.56	14
	Total MOAH	unspiked	<0.5	14
Cocoa butter	Total MOSH	unspiked	6.08	15
	Total MOAH	unspiked	<1.0	15

Test material

Matrix	Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Total number of results	Comparability: no. of results, which correspond to $ z\text{-score} \leq 2$	Trueness: no. of results, which correspond to recoveries of 70 to 120 % of the spiked level
Chocolate bar	Total MOSH	4.7	7.33*	14	11	10
	Total MOAH	4.1	3.27	14	13	12
Cocoa butter	Total MOSH	11	17.3*	15	15	15
	Total MOAH	5.3	5.38	15	11	13

* The levels of MOSH in the blank material are considered for evaluation.

The standardised analytical method DIN EN ISO 20122:2024 is applied by most of the labs even though it is developed for fats and oils. Effects of increasing standardisation and harmonisation were seen over the last years. The results of the laboratories became increasingly comparable. Even though the standardised method is 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. Applied methods are based on epoxidation with mCPBA or performic acid. Different solvents are used during epoxidation. The most common procedure of epoxidation is mCPBA in ethanol.

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 compare the outcome of the analytical methods of each individual laboratory 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 are:

- The choice of a suitable method for clean-up (e.g. aluminium oxide, silica gel, saponification, 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.

Expert knowledge is indispensable for a correct interpretation of the resulting chromatograms. The laboratories must be able to identify interferences to avoid misinterpretation and 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 is possible.