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Method ring test
MOSH/MOAH
in chocolate bread spread
P2205-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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Participants with any comments or concerns related to this ring test are invited to contact:

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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 exactly the same way as other participants and the same rules of confidentiality apply.

Method ring tests like P2205-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 complex and composite matrices like chocolate bread spread. Besides others, the method ring test shall answer the question whether the applied analytical methods are suitable for quantification of low levels of MOSH and MOAH in such matrices.

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 chocolate bread spread.

- 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.

A commercially available chocolate bread spread is chosen as matrices for the method ring test. An unspiked sample as well as a spiked sample of the bread spread are provided as blank material resp. test material. The chocolate bread spread is spiked with a technical white oil and a lubricant oil.

11 laboratories across four countries (Germany, Italy, Netherlands, and Switzerland) took part in the test. All laboratories reported results and are considered for evaluation. 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 material and the blank material.

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, 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 resp. total MOAH, and
b) total hump of total MOSH resp. total MOAH (according to JRC).

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

The blank material is free from MOAH (< 0.5 mg/kg), while it contains about 5 mg/kg MOSH. The test material is spiked with MOSH and MOAH. The levels of MOSH in the blank material are considered for evaluation of the trueness criterion related to the test material.

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 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. For evaluation of total MOSH, the results related to the blank material are subtracted from the results related to the test material.

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

Blank material

Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Total number of results
Total MOSH (total hump)	unspiked	5.15	11
Total MOAH (total hump)	unspiked	< 0.5	11

Test material

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
Total MOSH (total hump)	6.3	12.1*	11	10	8
Total MOAH (total hump)	4.4	3.73	10	6	6

* The assigned value corresponds to total MOSH in the blank material plus the spiked level.

Several approaches took place to harmonise the analytical methods, with focus on different groups of matrices (infant formula, fats, and oils, etc.). Analytical methods were improved to be able to quantify even low levels of MOSH and MOAH of about 1 mg/kg in matrices like edible oils. Clean-up procedures and especially the conditions for epoxidation were improved as well.

Still different approaches and concepts for clean-up and quantification/correction of measurements are applied by the laboratories. However, the level of harmonisation and the level of knowledge and experience of the labs improved a lot. Chromatograms are of much higher quality, compared to the first ring tests in 2019. And even if not the results of all labs are comparable and true, the overall performance of the labs improved a lot.

The assigned values are in good accordance with the spiked levels or with the expected concentration (total MOSH, spiked level plus MOSH in the blank material). 10 out of 11 labs pass the comparability criterion related to total MOSH, while 8 out of 11 labs pass the trueness criterion. Quantification of MOAH is more challenging. 6 out of 10 labs pass the comparability criterion as well as the trueness criterion.

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 methods 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?

In order to be able to produce reliable and true results, 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.

However, if the labs are experienced and sophisticated analytical methods are correctly applied, a reliable, comparable, and true quantification of MOSH and MOAH in complex and composite matrices like chocolate bread spread is possible, even at low levels.