Facing analytical quality.

Method ring test MOSH/MOAH in pepper (spice) P2103-MRT

Summary

The entire report is available to participants only.

Designed, realised and evaluated by

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Method ring tests like P2103-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 matrices like spices.

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 black pepper.

- 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 spiked sample of milled black pepper (spice) is provided as test material. Spiking is performed with the lubricant oil Shell Gadus and with a technical white oil. The corresponding unspiked milled black pepper is provided as blank material.

Eight laboratories across three countries (Germany, Italy, and Netherlands) took part in the test. All labs 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 and 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

and

- 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 (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).*

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. The lower bound results of total MOSH and total MOAH are provided for information only.

The blank material contains about 3 mg/kg of total MOSH, while it is free from total MOAH. The level of MOSH in the blank material is considered for the calculation of the recoveries of the spiked levels (trueness criterion). The difference between the results related to total MOSH the blank material and the results related to total MOSH the test material are calculated by PROOF-ACS for evaluation.

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 tables below:

Blank material

Parameter	Spiked level [mg/kg]	Assigned value [mg/kg]	Total number of results
Total MOSH (total hump)	unspiked	3.52	7
Total MOAH (total hump)	unspiked	< 1	2

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)	7.1	10.9	7	5	6
Total MOAH (total hump)	8.3	7.70	8	4	5

Up to now, no standardised analytical method is available for the quantification of MOSH and MOAH in spices. Consequently, different approaches and concepts for clean-up are applied by the participants. 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. If the same type of clean-up is applied, the procedures might be rather different in detail.

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 depending on the preferences of the individual labs.

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 last MOSH/MOAH method ring tests of PROOF (P1917-MRT, P1918-MRT, P2016-MRT), the performances of the laboratories improved a lot. Even though the matrix is quite challenging, most of the laboratories are able to perform a suitable clean-up by means of aluminium oxide, saponification resp. 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.

Five labs provided comparable results related to total MOSH, while six labs pass the trueness criterion related to total MOSH if the levels of MOSH in the blank material are considered for evaluation.

Four labs pass the comparability criterion related to total MOAH, while five labs pass the trueness criterion related to total MOAH.

The assigned values are close to the spiked levels for total MOSH (99 % recovery, if blank levels are considered) and total MOAH (93 % recovery).

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?

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.

The quantification of MOSH and MOAH remains challenging for complex matrices like spices. 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.