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Method ring test
MOSH/MOAH in infant formula
P2222-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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Method ring tests like P2222-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 infant formula. The question, whether or not the commonly applied analytical methods are suitable to quantify low levels of MOSH and MOAH in infant formula was discussed on a pan-European level in 2020/2021. As a consequence, this method ring test was organised to answer the question.

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 infant formula.

- 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 infant formula is provided as test material. The infant formula (powder) was not only spiked on the surface, but solved in water, spiked, freeze-dried and milled thereafter in order to achieve material, which is similar to commercially available infant formula. Spiking is performed with a technical white oil and with a technical creeping oil.

The corresponding unspiked infant formula, which is intensively homogenised manually without further sample preparation, is provided as blank material.

14 laboratories across four European countries (France, 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 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.

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 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 contains about 1 to 2 mg/kg of total MOSH, while it is free from total MOAH. The level of MOSH in the blank material is not considered 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 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 MOAH.

Evaluation of the trueness criterion was not applied to total MOSH. The assigned value is higher than expected based on the level of MOSH in the blank material and the spiked level. It cannot be excluded that the material was contaminated with MOSH or POSH during the advanced test material preparation procedure (solving the powder, spiking, freeze-drying, milling, homogenising). However, this does not influence the evaluation with respect to the comparability criterion.

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	2.23	9
Total MOAH (total hump)	unspiked	< 0.5	12

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)	13	19.6	13	9	Not evaluated
Total MOAH (total hump)	6.0	5.27	14	12	11

Up to now, no standardised analytical method is finalised for the quantification of MOSH and MOAH in infant formula. Consequently, different approaches and concepts for clean-up are applied by the participants. Aluminium oxide, epoxidation, saponification, and/or silica gel 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. A completely different analytical method was applied by one of the labs, which is not suitable for the analytical purpose.

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 first 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.

9 out of 14 labs provided comparable results related to total MOSH. 12 out of 14 labs pass the comparability criterion related to total MOAH, while 11 out of 14 labs pass the trueness criterion related to total MOAH.

Due to the satisfying performance of the labs, the assigned value related to total MOAH is close to the spiked level of 6 mg/kg (88 % recovery of the spiked level).

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.

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 infant formula is possible, even at low levels.