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# Method ring test
# MOSH/MOAH in infant formula (quantification)
# P2402-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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DAkkS
Deutsche
Akkreditierungsstelle
D-EP-22211-01-00

PROOF-ACS is a DAkkS accredited proficiency testing provider according to DIN EN ISO 17043:2010 (D-EP-22211-01-00). This method ring test is covered by the scope of accreditation.

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.

## Summary

Method ring tests are highly valuable to gather deep insights into the real challenges of complex analytical methods like the quantification of low levels of MOSH and MOAH in complex 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 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 quantification of MOSH and MOAH is based on the integration of the respective “humps”. The chromatograms of all laboratories are collected and summarised. Conspicuous chromatograms and pitfalls are discussed in the report.

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 to achieve material, which is like commercially available infant formula. Spiking is performed with a technical white oil and a lubricant.

The corresponding unspiked infant formula is provided as blank material. The raw material is intensively homogenised manually to prepare the blank material. No further sample preparation is performed.

10 laboratories across four European countries (France, Germany, Italy, and Netherlands) took part in the test. 9 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 and related to reagent blank samples.

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

According to the guidance document of the Joint Research Centre of the European Commission, 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 blank material is free from total MOAH (< 0.5 mg/kg), while it contains total MOSH at a concentration level about 2.1 mg/kg. The level of MOSH in the blank materials is considered during the evaluation of 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 *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. It is not applicable to total MOSH due to an assumed contamination of the test material with MOSH during the elaborated procedure of test material preparation (see above) and due to a level of MOSH in the raw 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 | unspiked | 2.13 | 9 |
| Total MOAH | unspiked | < 0.5 | - |

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 | 4.2* | 7.95 | 9 | 8 | Not applicable |
| Total MOAH | 3.8 | 3.82 | 9 | 8 | 8 |

* The spiked level is provided for information only. The blank material contains about 2.2 mg/kg of total MOSH and based on the assigned value, a contamination with about 1.5 mg/kg MOSH during the preparation of the test material a must be assumed.

Common proficiency tests are limited to the pure statistical evaluation of the provided data. The evaluation is mainly focussed on the comparability of results. However, the comparability is just a first step, much deeper insights are possible if the trueness criterion is applied.

And this method ring test goes even beyond the pure statistical evaluation of the data. The reported analytical results are combined with the applied analytical methods and the provided chromatograms.

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 condition for epoxidation. Furthermore, the method details can build the basis for further discussion and thus for a standardisation of the analytical methods.

The submitted chromatograms of all participants are summarised in part 3 of the report. Typical challenges related to chromatography are discussed in the report. Furthermore, the provided chromatograms allow to draw conclusions of probable problems of individual labs, which might cause over or underestimations of the true values. The chromatograms 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?

Several approaches took place to harmonise the analytical methods, which are applied for quantification of MOSH and MOAH throughout the last years. Different procedures for clean-up and epoxidation were tested intensively and improved. But still, the applied approaches and concepts for clean-up differ from lab to lab in detail. Aluminium oxide, epoxidation, saponification, and/or silica gel are chosen for clean-up depending in the preferences of the labs.

To summarise, the analytical methods were improved and LOQs were lowered to about 0.5 mg/kg for both, MOSH and MOAH and even for a complex matrix like infant formula. The overall quality of the submitted chromatograms improved and shows a much higher level of experience compared to earlier method ring tests.

The overall performance of the labs in this method ring test is satisfying. Most of the labs can provide comparable (and true) results related to MOSH and MOAH in infant formula at low concentration levels.