CHRONECT™ by TRAJAN

REPORT

MOSH/MOAH Analysis of SC PAFF- and JRC-Relevant Food Matrices from Low- to High-Fat Samples

CHRONECT™ Workstation MOSH/MOAH AWF – Sample and LOQ Determination

Overview of this report

This report documents the automated determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) in food matrices that are of regulatory interest under current SC PAFF and JRC recommendations, where proposed action limits and performance expectations are defined according to matrix fat content.

Three representative food matrices—olive oil, infant formula, and rice flour—were selected to cover low-, medium-, and high-fat categories as defined in these guidance documents. All analyses were performed using the CHRONECT Workstation MOSH/MOAH AWF (LC–GC–FID).

The report describes the sample preparation strategy, the fully automated processing sequence, and the resulting concentrations, precision, and detection capabilities for each matrix.

The first part of the report presents the analysis of real samples. For each matrix, it summarizes the experimental setup (sample weights, reagents, internal standards), the automated measurement sequence, and the results from five replicate injections plus blanks. Detailed tables provide internal standard and hump areas, calculated MOSH/MOAH concentrations (blank-corrected), retention times, and representative chromatograms.

The second part focuses on method performance in terms of detection and quantitation limits (LOD/LOQ). It explains the use of dedicated blank matrices, the statistical approach following Eurachem and JRC guidance, and the resulting LOD/LOQ values for all matrices. Spiking experiments with Gravex and SN 500 at realistic MOAH LOQ levels are reported to verify recovery and accuracy against JRC and SC PAFF recommendations.

Together, these sections demonstrate that the automated CHRONECT workflow delivers sensitive, precise, and traceable MOSH/MOAH measurements across SC PAFF/JRC-relevant fat categories, complies with ISO and JRC requirements, and can be independently verified by third-party laboratories.

Analysis of samples

Introduction

Why

Mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) remain a focus of regulatory attention due to their potential health relevance and their widespread occurrence in foodstuffs. Current guidance from the Joint Research Centre (JRC) and discussions within the Standing Committee on Plants, Animals, Food and Feed (SC PAFF) emphasize matrix-dependent action limits and performance requirements, with food categories differentiated according to fat content. Consequently, analytical methods must demonstrate reliable performance across low-, medium-, and high-fat matrices to support compliance assessment and enforcement.

What

In this study, three representative food matrices—olive oil, infant formula, and rice flour—were selected to reflect these fat-based categories as defined in JRC and SC PAFF recommendations. Olive oil represents a high-fat matrix, infant formula a medium-fat matrix, and rice flour a low-fat matrix. The selection enables a systematic evaluation of method robustness, sensitivity, and quantitation capability under matrix conditions that are directly relevant for current and future regulatory expectations on MOSH/MOAH.

How

Each sample (1 or 2.5 g) was prepared and analyzed using a CHRONECT Workstation MOSH/MOAH AWF, a fully automated LC–GC–FID system designed for standardized MOSH/MOAH determination. Sample preparation involved the addition of hexane, ethanol, aqueous KOH, and an internal standard to each weighed sample. These additions were performed manually—using a solvent dispenser for organic solvents and a pipette for KOH—to accelerate sample throughput, avoid potential corrosive damage to robotic components, and prevent septum puncture–related losses of the internal standard. Although the workstation can be configured to automate these steps, manual execution was selected as the most practical and robust approach for this application.

The workstation can also be operated as a stand-alone system without LC–GC–FID injection if required. All consumables were prepared in a professional and application-appropriate manner, and the laboratory environment was designed to be optimized for MOSH/MOAH analysis.

A Restek internal standard (cat. #31070) was diluted 1:50, and 50 µL (corresponding to 1500 ng) was added to each sample. Table 1 summarizes the sample weights and reagent volumes used for each matrix.

Table 1. Sample preparation: weighed sample and added reagents. (Internal standard Restek #31070, 1:50 dilution; 50 µL =1500 ng per sample).

Matrix	Sample weight (g)	Hexane (mL)	Ethanol (mL)	KOH (mL)	Internal standard (µL)
Olive oil	1.00	10	3	2	50
Infant formula	1.00	10	3	2	50
Rice flour	2.50	8	3	-	50

A total of 18 samples (5 replicates + 1 blank per matrix) were prepared in three batches (one batch per matrix), along with corresponding chemical blanks. Each sample was weighed to two decimal places to avoid mix-ups.

Automated Preparation and Analysis

Following manual reagent addition, the CHRONECT Workstation executed fully automated sample preparation and measurement using the CHRONECT MOSH/MOAH Automated Workflow. Olive oil and infant formula samples were first subjected to alkaline saponification, followed by two sequential extractions and epoxidation with performic acid in 1-chlorobutane prior to analysis by a two-channel LC–GC–FID system. For olive oil, an additional on-line alumina (Al_2O_3) cleanup step was applied in accordance with ISO 20122:2024.

Rice flour samples were prepared by two extractions with hexane and subsequently analyzed using the same two-channel LC–GC–FID configuration. After sample loading, all processing and analytical steps were carried out without any further manual intervention.

Measurement Sequence (Figure 1): All 18 samples were processed in one continuous sequence. Five replicates of each matrix (plus one blank) were analyzed in batch mode. While the first batch was being prepared, the system automatically injected four performance-check standards for verification. Then all sample injections proceeded automatically. The entire sequence (18 samples) was completed in under 16 hours.

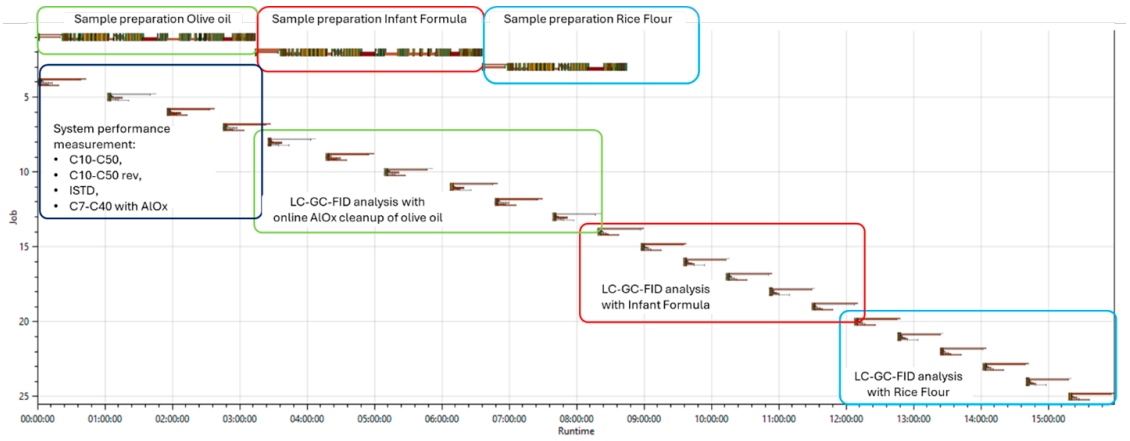

Figure 1. Measurement sequence of the MOSH/MOAH analysis (each matrix was run in a separate batch; five replicates + one blank each). During the first batch, four standard checks were automatically injected to monitor system performance.

Results

Summary: Below is a concise summary of the five-replicate results for each sample matrix. The table above shows mean concentrations and the relative standard deviation (RSD %) calculated from the five replicate analyses (n = 5). All values reported are blank-corrected (chemical blank from the sample preparation step was subtracted).

In the sections that follow, each sample matrix (olive oil, infant formula, rice flour) is presented in detail: individual replicate data (peak areas, ISTD values, hump areas), verification ratios, retention times, LOQ/LOD information and labelled chromatograms. The full measurement reports for each injection — including raw chromatograms, instrument logs and processed integration files — are provided with this report as supporting documentation and are available for independent review or reprocessing by a third-party laboratory.

Matrix/ Sample	MOSH Average [mg/kg]	MOSH RSD [%]	MOAH Average [mg/kg]	MOAH RSD [%]
Olive oil	63.47	1.1	4.22	2.5
Infant formula	4.53	1.8	1.36	4.5
Rice flour	3.10	0.7	0.51	2.9

Olive Oil

MOSH (Mineral Oil Saturated Hydrocarbons) – Olive Oil

The following tables summarize the internal-standard peak areas, MOSH hump areas, calculated concentrations, and retention times for olive oil (blank and 5 replicates). The chemical blank (preparation reagents without sample) is included. All concentrations are blank-corrected (chemical blank subtracted) as noted.

Table 2. Olive oil MOSH analysis: peak areas of internal standards (C11, CyCy, C13, Cho) and the MOSH "hump" for blank and 5 replicates.

Area	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
C11	1,369,793	1,844,754	1,589,939	1,951,988	2,474,579	1,155,720
CyCy	1,670,008	2,003,572	1,561,151	1,831,766	2,307,180	1,327,690
C13	891,251	1,187,780	935,676	1,088,422	1,382,443	786,007
Cho	130,513	196,930	133,235	164,720	227,343	69,371
Hump (MOSH fraction)	213,761	85,026,242	65,437,195	78,271,354	99,502,526	55,770,962

Table 3. Olive oil MOSH results: input parameters and calculated MOSH concentration for each replicate (chemical blank corrected; see Note).

Parameter	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Sample weight (g)	1.00	1.00	1.00	1.00	1.00	1.00
Internal Std (ISTD) [mg/kg]	1.50	1.50	1.50	1.50	1.50	1.50
MOSH [mg/kg]	0.19	63.66	62.87	64.10	64.69	63.01
MOSH (blank-corrected)* [mg/kg]	-	63.46	62.68	63.90	64.50	62.82

**Note: MOSH values have been corrected by subtracting the chemical blank (shown as "blank-corrected" above).*

Table 4. Olive oil MOSH hump retention times (start, apex, end). (Blank = no hump detected.)

Retention Times (MOSH hump)	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Hump start [min]	-	11.3	11.3	11.3	11.3	11.3
Hump apex [min]	-	16.5	16.4	16.4	16.4	16.4
Hump end [min]	-	23.4	23.4	23.4	23.4	23.5

Table 5. Olive oil MOSH LOQ (JRC/Eurachem guideline) for each replicate (blank and samples).

LOQ (JRC/Eurachem) [mg/kg]	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
LOQ (JRC/Eurachem)*	0.23	0.23	0.23	0.23	0.23	0.23

**LOQ (lowest limit of quantification) is based on JRC/Eurachem criteria (details in "Detection and Quantitation Limits" below).*

MOAH (Mineral Oil Aromatic Hydrocarbons) – Olive Oil

Table 6–7 show the MOAH results for olive oil (blank + 5 replicates). The on-line Al_2O_3 cleanup reduces long-chain biogenic n-alkanes.

Table 6. Olive oil MOAH analysis: peak areas of ISTDs (5B, 2MN, 1MN, TBB, Per) and MOAH hump (blank + 5 replicates).

Area	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
5B	1,628,643	1,912,395	1,413,438	1,710,673	2,099,156	1,182,048
2MN	1,665,188	1,879,653	1,427,052	1,696,728	2,142,229	1,195,070
1MN	1,816,438	2,039,711	1,547,545	1,834,637	2,336,644	1,303,556
TBB	1,849,649	1,770,069	1,676,450	2,001,154	2,589,538	1,422,608
Per	776,171	2,028,676	1,977,436	1,819,229	2,798,869	1,455,968
Hump (MOAH fraction)	123,310	4,896,012	4,815,881	5,684,613	7,663,305	4,191,004

Table 7. Olive oil MOAH results: input parameters and calculated MOAH concentration for each replicate (blank-corrected).
**Note: MOAH values have been corrected by subtracting the chemical blank (shown as "blank-corrected" above).*

Parameter	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Sample weight (g)	1.00	1.00	1.00	1.00	1.00	1.00
ISTD [mg/kg]	1.50	1.50	1.50	1.50	1.50	1.50
MOAH [mg/kg]	0.10	4.15	4.31	4.26	4.44	4.42
MOAH (blank-corrected)* [mg/kg]	-	4.05	4.21	4.16	4.34	4.32

Table 8. Olive oil MOAH hump retention times.

LOQ (lowest limit of quantification) is based on JRC/Eurachem criteria (details in "Detection and Quantitation Limits" below).

LOQ (JRC/Eurachem) [mg/kg]	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
LOQ (JRC/Eurachem)*	0.06	0.06	0.06	0.06	0.06	0.06

Chromatograms (Figures 2–4): Figure 2 shows the five overlaid MOAH chromatograms of the olive oil replicates (very consistent). Figures 3–4 show the individual MOSH and MOAH chromatograms for Replicate 1 (with 63.66 mg/kg MOSH and 4.15 mg/kg MOAH) as representative examples.

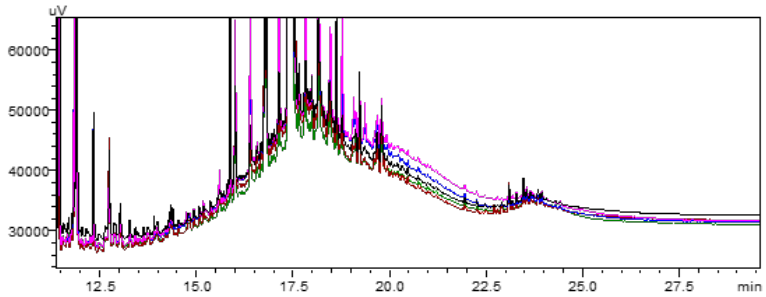

Figure 2. Overlay of the MOAH chromatograms for all 5 olive oil replicates (Rep 1–5). The uniform "hump" indicates high reproducibility of the method.

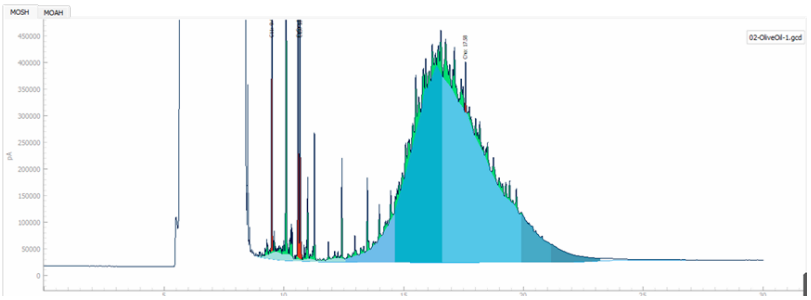

Figure 3. MOSH chromatogram (LC–GC–FID) for olive oil Replicate 1 (63.66 mg/kg MOSH).

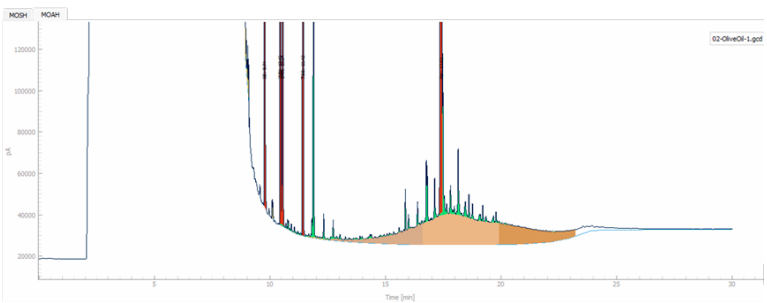

Figure 4. MOAH chromatogram for olive oil Replicate 1 (4.15 mg/kg MOAH; hump only)

Infant Formula

MOSH – Infant Formula

Tables 10–12 present the results for the infant formula (blank + 5 replicates).

Table 10. Infant formula MOSH: peak areas of ISTDs and MOSH hump (blank + 5 replicates).

Area	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
C11	1,568,815	1,833,303	2,107,029	1,499,689	2,038,685	1,294,747
CyCy	1,930,717	2,173,106	2,553,115	1,791,203	2,507,129	1,492,390
C13	992,063	1,118,109	1,305,791	922,343	1,274,064	765,134
Cho	4,300,463	5,446,425	6,284,078	4,395,681	6,141,679	3,787,557
Hump (MOSH fraction)	243,270	6,848,183	7,776,789	5,716,325	8,019,470	4,677,150

Table 11. Infant formula MOSH results (blank-corrected).

Parameter	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Sample weight (g)	1.00	1.00	1.00	1.00	1.00	1.00
Internal Std (ISTD) [mg/kg]	1.50	1.50	1.50	1.50	1.50	1.50
MOSH [mg/kg]	0.19	4.73	4.57	4.79	4.80	4.70
MOSH (blank-corrected)* [mg/kg]	-	4.54	4.38	4.60	4.61	4.51

Table 12. Infant formula MOSH hump retention times.

Retention Times (MOSH hump)	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Hump start [min]	-	12.6	12.5	12.5	12.5	12.5
Hump apex [min]	-	16.0	15.9	15.9	15.9	15.9
Hump end [min]	-	22.6	22.7	22.7	22.7	22.7

Table 13. Infant formula MOSH LOQ (JRC/Eurachem).

LOQ (JRC/Eurachem) [mg/kg]	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
LOQ (JRC/Eurachem)*	0.06	0.06	0.06	0.06	0.06	0.06

MOAH – Infant Formula

Tables 14-15 summarize the infant formula MOAH results.

Table 14. Infant formula MOAH analysis: peak areas of ISTDs (5B, 2MN, 1MN, TBB, Per) and MOAH hump (blank + 5 replicates).

Area	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
5B	1,853,406	2,098,857	2,408,210	1,710,830	2,193,302	1,389,653
2MN	1,948,989	2,173,866	2,503,560	1,767,313	2,348,905	1,465,169
1MN	2,129,665	2,371,850	2,713,863	1,929,558	2,570,235	1,601,765
TBB	2,177,215	2,425,589	2,830,653	1,968,494	2,689,021	1,650,441
Per	860,811	3,272,036	3,787,429	2,393,785	3,333,875	2,039,922
Hump (MOAH fraction)	94,346	2,221,839	2,634,395	1,830,699	2,685,435	1,650,441

Table 15. Infant formula MOAH results (blank-corrected).

Parameter	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Sample weight (g)	1.00	1.00	1.00	1.00	1.00	1.00
ISTD [mg/kg]	1.50	1.50	1.50	1.50	1.50	1.50
MOAH [mg/kg]	0.10	1.37	1.35	1.35	1.35	1.35
MOAH (blank-corrected)* [mg/kg]	-	1.25	1.25	1.25	1.25	1.25

**Note: MOAH values have been corrected by subtracting the chemical blank (shown as "blank-corrected" above).*

Table 16. Infant formula MOAH hump retention times.

Retention Times (MOSH hump)	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Hump start [min]	-	12.6	12.5	12.5	12.5	12.5
Hump apex [min]	-	16.0	15.9	15.9	15.9	15.9
Hump end [min]	-	22.6	22.7	22.7	22.7	22.7

Table 17. Infant formula MOAH LOQ (JRC/Eurachem).

LOQ (JRC/Eurachem) [mg/kg]	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
LOQ (JRC/Eurachem)*	0.06	0.06	0.06	0.06	0.06	0.06

**LOQ (lowest limit of quantification) is based on JRC/Eurachem criteria (details in "Detection and Quantitation Limits" below).*

Chromatograms (Figures 5–7): Figure 5 shows the overlay of the 5 infant formula MOAH chromatograms (1.35 mg/kg average), illustrating excellent reproducibility. Figures 6–7 display the Replicate 1 MOSH (4.72 mg/kg) and MOAH (1.35 mg/kg) chromatograms.

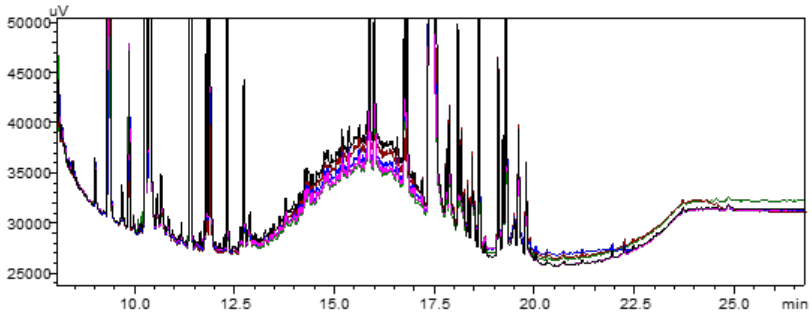

Figure 5. Overlay of the MOAH chromatograms for all 5 infant formula replicates (1.35 mg/kg MOAH). Peaks and hump align closely, showing high reproducibility.

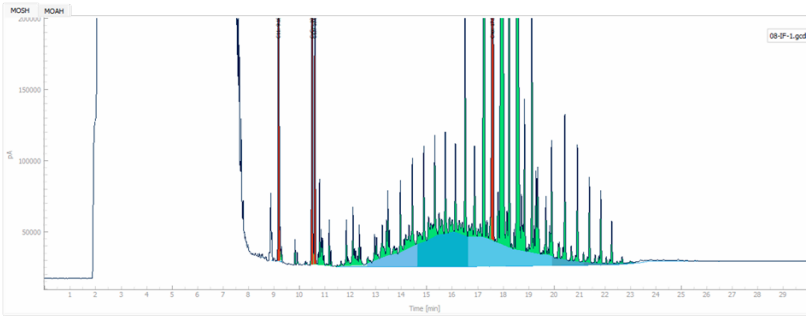

Figure 6. MOSH chromatogram (LC–GC–FID) for infant formula Replicate 1 (4.72 mg/kg)

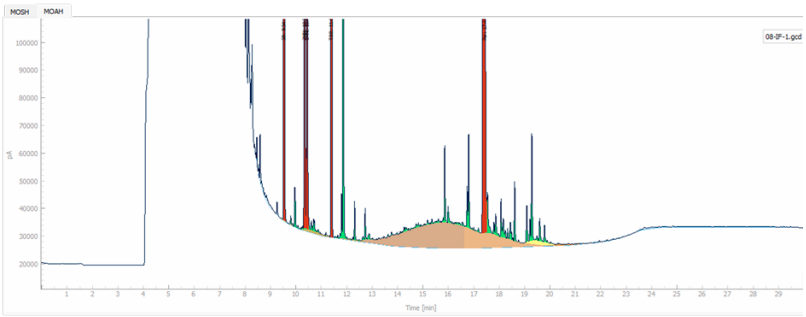

Figure 7. MOAH chromatogram for infant formula Replicate 1 (1.35 mg/kg MOAH).

Rice Flour

MOSH – Rice Flour

Tables 18–20 present the rice flour results.

Table 18. Rice flour MOSH: ISTD and hump areas.

Area	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
C11	3,402,620	3,401,843	1,814,217	3,082,515	3,244,202	2,417,996
CyCy	3,819,437	3,733,560	2,117,658	3,472,600	3,647,953	2,725,359
C13	1,919,195	1,886,222	1,075,945	1,756,731	1,838,006	1,374,238
Cho	8,092,376	8,388,177	4,797,976	7,783,972	8,129,785	5,959,356
Hump (MOSH fraction)	267,361	19,433,181	11,001,232	18,167,484	19,139,594	14,417,148

Table 19. Rice flour MOSH results (blank-corrected).

Parameter	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Sample weight (g)	2.50	2.50	2.50	2.50	2.50	2.50
Internal Std (ISTD) [mg/kg]	1.50	1.50	1.50	1.50	1.50	1.50
MOSH [mg/kg]	0.04	3.12	3.12	3.14	3.15	3.17
MOSH (blank-corrected)* [mg/kg]	-	3.08	3.08	3.10	3.11	3.13

Table 20. Rice flour MOSH hump retention times.

Retention Times (MOSH hump)	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
1. Hump start [min]	-	11.4	11.5	11.3	11.4	11.4
1. Hump apex [min]	-	16.2	16.2	16.2	16.2	16.2
1. Hump end [min]	-	21.0	20.9	21.1	21.0	21.0
2. Hump start [min]	-	13.9	14.0	13.9	13.9	13.9
2. Hump apex [min]	-	19.4	19.4	19.4	19.4	19.4
2. Hump end [min]	-	24.9	24.8	24.9	24.9	24.9

Note: MOSH values have been corrected by subtracting the chemical blank (shown as "blank-corrected" above).

Table 20. Rice flour MOSH hump retention times.

LOQ (JRC/Eurachem) [mg/kg]	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
LOQ (JRC/Eurachem)*	0.28	0.28	0.28	0.28	0.28	0.28

Note: There are two humps in the chromatogram. This means that the end time of the first hump and the start time of the second hump cannot be identified because they overlap. Therefore, the times were calculated under the assumption of a symmetrical Gaussian distribution of the two humps.

MOAH – Rice Flour

Tables 22–23 show the rice flour MOAH results.

Table 22. Rice flour MOAH: ISTD and hump areas.

Area	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
5B	3,856,669	3,757,230	1,944,520	3,471,900	3,577,410	2,625,963
2MN	3,955,342	3,869,581	2,118,947	3,589,237	3,747,869	2,756,254
1MN	4,206,645	4,124,008	2,242,404	3,822,089	3,997,674	2,926,048
TBB	4,202,380	4,114,097	2,370,333	3,830,836	4,022,499	2,964,946
Per	8,591,939	6,864,270	4,871,865	6,907,435	6,624,400	6,044,527
Hump (MOAH fraction)	210,119	3,867,251	2,109,596	3,409,444	3,720,811	2,589,386

Table 23. Rice flour MOAH results (blank-corrected).

Parameter	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Sample weight (g)	2.50	2.50	2.50	2.50	2.50	2.50
ISTD [mg/kg]	1.50	1.50	1.50	1.50	1.50	1.50
MOAH [mg/kg]	0.03	0.56	0.53	0.53	0.56	0.52
MOAH (blank-corrected)* [mg/kg]	-	053	0.50	0.50	0.53	0.49

Note: MOAH values have been corrected by subtracting the chemical blank (shown as "blank-corrected" above).

Table 24. Rice flour MOAH hump retention times.

Retention Times (MOSH hump)	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
Hump start [min]	-	13.9	14.0	13.9	13.9	13.9
Hump apex [min]	-	19.4	19.4	19.4	19.4	19.4
Hump end [min]	-	24.9	24.8	24.9	24.9	24.9

Table 25. Rice flour MOAH LOQ (JRC/Eurachem).

LOQ (JRC/Eurachem) [mg/kg]	Blank	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
LOQ (JRC/Eurachem)*	0.06	0.06	0.06	0.06	0.06	0.06

LOQ (lowest limit of quantification) is based on JRC/Eurachem criteria (details in "Detection and Quantitation Limits" below).

Chromatograms (Figures 8–10): Figure 8 overlays the 5 rice flour MOAH chromatograms (average 0.51 mg/kg). Figures 9–10 show Replicate 1 MOSH (3.10 mg/kg) and MOAH (0.56 mg/kg) chromatograms.

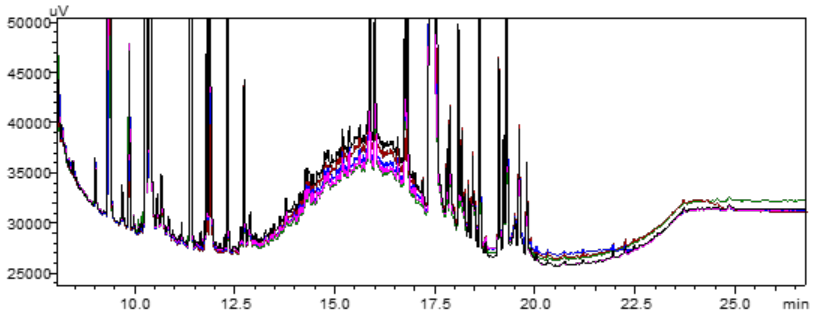

Figure 8. Overlay of the MOAH chromatograms for all 5 rice flour replicates (0.51 mg/kg MOAH).

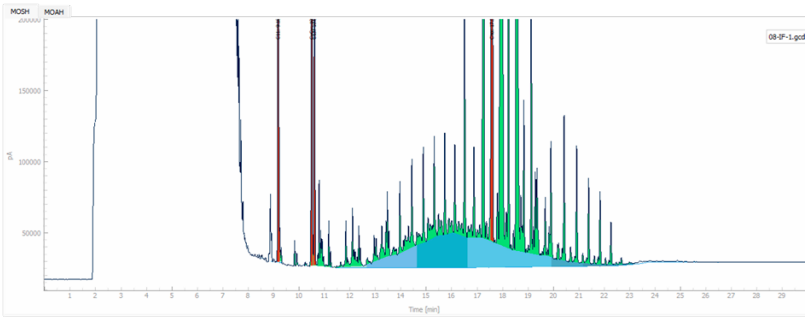

Figure 9. MOSH chromatogram (LC–GC–FID) for rice flour Replicate 1 (3.10 mg/kg MOSH).

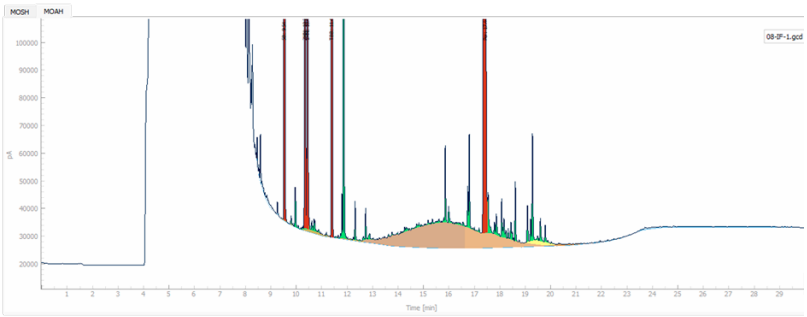

Figure 10. MOAH chromatogram for rice flour Replicate 1 (0.56 mg/kg MOAH).

Detection and Quantitation Limits (LOD/LOQ)

Introduction

For the determination of method detection and quantitation limits (LOD/LOQ), dedicated blank matrices were used. These blank matrices were procured specifically for validation. Using independent blank matrices avoids bias from the test samples, provides a well-characterized baseline for the automated preparation workflow, and supports reproducibility and traceability of the LOD/LOQ results.

Matrix type	Source/material	Identifier/supplier	Notes/purpose
Olive oil (extra virgin)	Retail trade	Commercial product	Purchased commercial oil used as oil-type blank matrix for MOSH/MOAH LOD/LOQ measurements.
Infant formula (blank)	Proof-ACS	P2402-BLIf	Certified blank/reference matrix used as a dairy/infant formula blank for LOD/LOQ and precision testing.
Rice flour (blank)	Proof-ACS	P2503-BLFi	Certified blank/reference matrix used as a low-fat/dry food blank for LOD/LOQ and precision testing.

Preparation

Each sample (1 or 2.5 g) was prepared and measured on a CHRONECT Workstation MOSH/MOAH AWF (fully automated LC–GC–FID system). Sample preparation involved adding hexane, ethanol, aqueous KOH, and an internal standard to each weighed sample. These additions were performed manually (with a dispenser for solvents and a pipette for KOH) to speed up the process, avoid corrosive damage to the robot, and prevent septum puncture losses of the internal standard. Although the workstation can be expanded to automate these additions as well, we recommend carrying out these particular steps manually, as the practical benefits clearly outweigh the disadvantages. The workstation can also operate as a stand-alone device without LC–GC–FID injection if required.

Restek internal standard (cat. #31070) was diluted 1:50, and 50 µL (1500 ng) was added to each sample. Table 1 summarizes the sample weights and reagent volumes for each matrix. Each sample was weighed to two decimal places to avoid mix-ups.

Table 1. Sample preparation: weighed sample and added reagents. (Internal standard Restek #31070, 1:50 dilution; 50 µL =1500 ng per sample).

Matrix	Sample weight (g)	Hexane (mL)	Ethanol (mL)	KOH (mL)	Internal standard (µL)
Olive oil	1.00	10	3	2	50
Infant formula	1.00	10	3	2	50
Rice flour	2.50	8	3	–	50

Automated Preparation and Analysis

After manual reagent addition, the CHRONECT Workstation carried out fully automated sample preparation and measurement. Olive oil and infant formula samples were first saponified (alkaline digestion), then each was extracted twice and epoxidized (with performic acid in 1-chlorobutane) before analysis by a two-channel LC–GC–FID. The olive oil additionally underwent an on-line alumina (Al_2O_3) cleanup step, as specified by ISO 20122:2024 for this matrix. The rice flour was prepared by two extractions with hexane and then analyzed on the two-channel LC–GC–FID. All processing steps ran without manual intervention after loading the samples.

LOD/LOQ determination

The detection limit (LOD) and quantitation limit (LOQ) were determined according to standard guidelines (Eurachem Guide: The Fitness for Purpose of Analytical Methods – A Laboratory Guide to Method Validation and Related Topics, (3rd ed. 2025)). Blank matrices (olive oil, infant formula, rice flour) were each measured 10 times on the same fully-automated system. The LOD was calculated as:

$$LOD = 3 \times SD \times \frac{1}{\sqrt{n}}$$

where SD is the standard deviation of the blank measurements and n the number of repeats. The LOQ was set at 3.3×LOD in line with JRC guidance (Guidance on sampling, analysis and data reporting for the monitoring of mineral oil hydrocarbons in food and food contact materials - 2nd Edition). Table 26 below summarizes the replicate results, averages, and resulting LOD/LOQ for each matrix.

Table 26. Blank-matrix replicate measurements (Olive oil, Infant formula, Rice flour; n=10) and resulting limits. Averages and standard deviations (SD) are shown. LOD = 3·SD/√n, LOQ = 3.3·LOD.

Sample no	Olive oil mg/kg		Infant formula (mg/kg)		Rice flour (mg/kg)	
	MOSH	MOAH	MOSH	MOAH	MOSH	MOAH
1	1.572	0.126	2.423	0.124	2.048	0.148
2	1.351	0.053	2.357	0.134	2.335	0.136
3	1.486	0.095	2.370	0.149	2.148	0.179
4	1.515	0.120	2.179	0.163	2.190	0.127
5	1.486	0.102	2.509	0.106	2.175	0.103
6	1.662	0.110	2.225	0.104	2.153	0.131
7	1.486	0.091	2.298	0.161	2.131	0.140
8	1.476	0.084	2.413	0.105	2.098	0.100
9	1.503	0.105	2.346	0.149	2.039	0.183
10	1.478	0.103	2.408	0.096	2.011	0.123
Average	**1.502**	**0.099**	**2.353**	**0.129**	**2.133**	**0.137**
SD	0.074	0.019	0.093	0.024	0.089	0.026
RSD%^	5.0	19.6	3.9	18.7	4.2	19.1
LOD	0.07	0.02	0.09	0.02	0.08	0.02
LOQ	0.23	0.06	0.29	0.08	0.28	0.08

The determined limits are very low (≤0.09 mg/kg for MOSH and ≤0.02 mg/kg for MOAH, LOD; LOQ ≤0.29 mg/kg MOSH and ≤0.08 mg/kg MOAH). This reflects the excellent reproducibility of the automated preparation. Figure 11 (below) illustrates reproducibility by overlaying all 10 MOAH chromatograms of the infant formula blank (0.129 mg/kg): the traces are essentially identical.

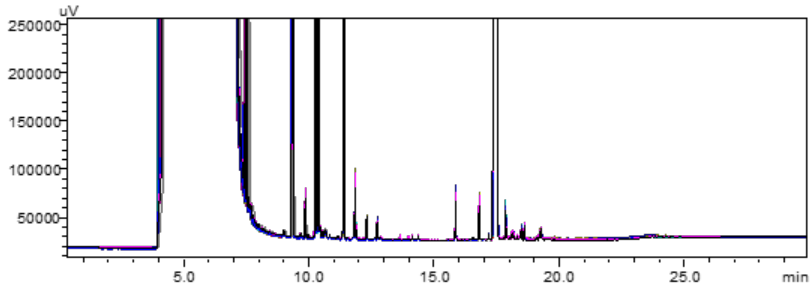

Figure 11. Overlay of the 10 replicate MOAH chromatograms of the infant formula blank (0.129 mg/kg average). The nearly identical patterns indicate very low variability.

Spiking Experiments (LOQ Verification)

Since the MOAH LOD/LOQ values are extremely low (<0.08 mg/kg), far below relevant contamination levels, method sensitivity was verified by spiking at realistic LOQs. The JRC Technical Guide and EU SC PAFF committee specify MOAH LOQs depending on fat content. Based on these, the blank matrices were categorized by fat content and spiked at half the recommended LOQ with two mineral oil standards: Gravex (72.7% MOSH, 26.0% MOAH) and SN 500 (60.1% MOSH, 34.5% MOAH). Tables 27–28 summarize the spiking results.

The JRC/SC-PAFF recommended MOAH LOQs by food category are:

- Dry foods (≤ 4 % fat): MOAH LOQ = **0.5 mg/kg**
  → spike level used = **0.25 mg/kg** (½ JRC LOQ)

- Foods (>4 % fat, ≤50 % fat): MOAH LOQ = **1.0 mg/kg**
  → spike level used = **0.50 mg/kg** (½ JRC LOQ)

- Fats & oils (>50 % fat): MOAH LOQ = **2.0 mg/kg**
  → spike level used = **1.00 mg/kg** (½ JRC LOQ)
-

Table 27. Spiking of blank matrices at realistic MOAH LOQs with Gravex (upper section) and SN 500 (lower). The recovery is (measured – blank)/added ×100%. All recoveries are within 96–119% (JRC recommended range: 70-120%).

	Olive oil mg/kg		Infant formula (mg/kg)		Rice flour (mg/kg)	
	MOSH (mg/kg)	**MOAH (mg/kg)**	**MOSH (mg/kg)**	**MOAH (mg/kg)**	**MOSH (mg/kg)**	**MOAH (mg/kg)**
Gravex spike (0.5 mg/kg MOAH)						
Measured (spiked)	1.50	0.10	2.35	0.13	2.13	0.14
Gravex added (0.5 mg/kg MOAH)	**2.79**	**1.00**	**1.39**	**0.50**	**0.70**	**0.25**
Measured (spiked)	4.53	1.28	3.83	0.69	2.89	0.43
Recovery [%]	**109**	**119**	**106**	**111**	**109**	**116**

	Olive oil mg/kg		Infant formula (mg/kg)		Rice flour (mg/kg)	
	MOSH (mg/kg)	**MOAH (mg/kg)**	**MOSH (mg/kg)**	**MOAH (mg/kg)**	**MOSH (mg/kg)**	**MOAH (mg/kg)**
SN500 spike (0.5 mg/kg MOAH)						
Measured (spiked)	1.50	0.10	2.35	0.13	2.13	0.14
SN500 added (0.5 mg/kg MOAH)	**1.75**	**1.00**	**0.87**	**0.50**	**0.44**	**0.25**
Measured (spiked)	3.18	1.07	3.32	0.67	2.63	0.44
Recovery [%]	**96**	**96**	**111**	**107**	**114**	**119**

Figure 12–14 show one set of MOAH chromatograms for each matrix: blank (black) vs blank spiked with Gravex (pink) and SN 500 (blue). The spiked peaks are clearly above the unspiked baseline.

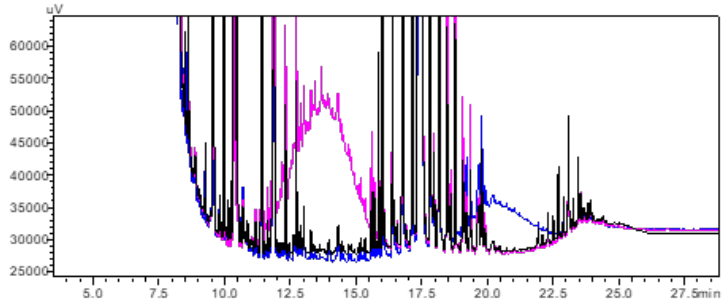

Figure 12. Olive oil MOAH chromatograms: blank (black), +1 mg/kg Gravex (pink), +1 mg/kg SN 500 (blue). Both spikes show clear signals above the blank hump.

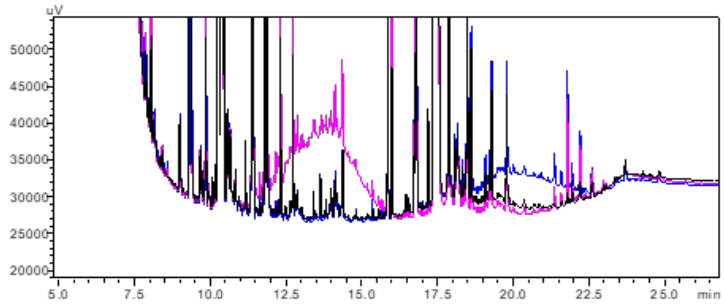

Figure 13. Infant formula MOAH chromatograms: blank (black), +0.5 mg/kg Gravex (pink), +0.5 mg/kg SN 500 (blue). The spiked peaks stand out from the blank.Table 27. Spiking of blank matrices at realistic MOAH LOQs with Gravex (upper section) and SN 500 (lower). The recovery is (measured – blank)/added ×100%. All recoveries are within 96–119% (JRC recommended range).

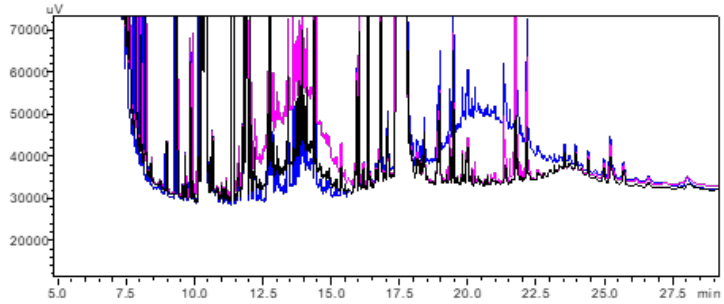

Figure 14. Rice flour MOAH chromatograms: blank (black), +0.25 mg/kg Gravex (pink), +0.25 mg/kg SN 500 (blue). Spiked MOAH (blue, pink) are distinct from the blank.

All recoveries (96–119%) meet JRC criteria, confirming the method's accuracy at low contamination levels.

Conclusion on LOQ Performance

Using the fully automated CHRONECT Workstation MOSH/MOAH AWF, we achieved MOSH/MOAH quantitation with LOQs roughly one-half of the regulatory requirements (JRC/SC PAFF) for the three product categories. Recovery tests with reference oils (Gravex, SN 500) at realistic LOQs showed excellent agreement (96–119%). Thus, the described method meets all ISO and JRC guidelines for MOSH/MOAH analysis.

Additional Information (Reference Materials)

Certified reference materials for infant formula and rice flour were used to determine the LOQ (Proof-ACS P2402-BLIf and P2503-BLRi). The average of the five replicates for these samples agrees with the certified values:

Table 28. Comparison of our measured averages (MOSH/MOAH) to certified values for infant formula (P2402-BLIf) and rice flour (P2503-BLRi). The results match the reference within expected uncertainty.

	MOSH [mg/kg]	MOAH [mg/kg]	MOSH [mg/kg]	MOAH [mg/kg]
Measured average	2.35	0.13	2.13	0.14
Proof-ACS cert	2.1	<0.5	2.1	<0.3

These results confirm that the automated MOSH/MOAH method is accurate and traceable. All analytical details (sample prep, conditions, validation) are fully documented above to allow independent verification by any third-party laboratory.

Final conclusion

This study demonstrates that the fully automated CHRONECT Workstation MOSH/MOAH AWF enables robust, precise, and regulatorily relevant MOSH/MOAH analysis across food matrices covering low-, medium-, and high-fat categories as defined by JRC and SC PAFF guidance. Consistent results were obtained for olive oil, infant formula, and rice flour, with low relative standard deviations in five-replicate measurements.

Method performance in terms of detection and quantitation limits met and exceeded current regulatory expectations, achieving LOQs one-half of the levels proposed by JRC and SC PAFF for the respective matrix categories.

Overall, the automated CHRONECT workflow complies with ISO and JRC requirements, provides fully traceable and reproducible results, and supports independent verification by third-party laboratories, making it a reliable solution for routine MOSH/MOAH analysis in regulatory-relevant food matrices.

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