Short note

Determination of Mineral Oil Aromatic Hydrocarbons (MOAH) in vegetable oils and fats - Comparison between RP-HPLC- Fluo and LC-GC-FID method

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This study provides an update on the application of the RP-HPLC-FLUO method for MOAH determination, following the previously published work on its validation and applicability [1]. A total of 161 edible oil samples from 10 different matrices were analysed in 2024 and compared with LC-GC-FID results [2]. The methods showed good alignment, particularly in sunflower oils, while grapeseed and rice oils confirmed higher contamination levels. Five additional samples from interlaboratory circuits validated the method's accuracy. RP-HPLC-FLUO is suggested for use as a valuable semi-quantitative tool for pre-screening analysis, suitable for routine monitoring and high-throughput control strategies.

Keywords: MOAH, HPLC-FLUO, Contaminants, LC-GC-Flm

1. INTRODUCTION

Mineral oil aromatic hydrocarbons (MOAH) are a class of complex aromatic compounds that originate from petroleum refining. MOAH have known concerns due to their potential genotoxic and carcinogenic effects, especially those containing multiple aromatic rings. Their presence in food matrices, particularly edible oils, has drawn increasing regulatory and scientific attention due to possible contamination during processing, storage, and packaging. While the reference-standard method for MOAH determination - on-line LC-GC-FID as per EN ISO 16995 and its subsequent revision method UNI EN ISO 20122:2024 [2] - offers high sensitivity and specificity, it remains analytically demanding, requiring dedicated instrumentation, advanced purification steps, and significant operational costs. The JRC technical report [3] points out that the analytical method used must be sufficiently sensible to ensure that food is not contaminated with potentially carcinogenic MOAH and that any other technique is acceptable only if it provides equivalent results to LC-GC-FID. In response to these challenges, an alternative method using reverse phase high-performance liquid chromatography coupled with fluorescence detection (RP-HPLC-FLUO) was recently proposed and validated as a rapid, cost-effective, and accessible screening technique for routine MOAH monitoring [1]. This method leverages the intrinsic fluorescent properties of aromatic compounds to offer semi-quantitative estimation with sufficient sensitivity and repeatability for preliminary risk assessment, as validated through international proficiency testing and inter-laboratory comparisons.
The present work provides an extensive update on the application of the HPLC-FLUO method to a large and diversified sample set (n=161) collected during 2024. A total of 10 distinct matrices were considered, including crude and refined oils from sunflower, grapeseed, rice, and other seeds. For each sample, MOAH levels were determined using both HPLC-FLUO and LC-GC-FID, allowing direct method comparison.

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The results demonstrate that HPLC-FLUO yields MOAH quantifications in close alignment with those from the reference LC-GC-FID method across most matrices, particularly in sunflower seed oil, which represented approximately half of the dataset. Notably, grapeseed and rice oils consistently showed the highest MOAH levels, corroborating findings from previous studies and highlighting these matrices as particularly prone to contamination. Some matrix-dependent deviations were observed, reflecting differences in chemical complexity and possible interferences, without compromising the screening utility of HPLC-FLUO.
Furthermore, this study evidenced a consistent alignment between the results obtained by HPLC-Fluo and those achieved by LC-GC-FID, as corroborated through their comparison across multiple proficiency tests.
Overall, this comparative study reaffirms the applicability of the HPLC-FLUO method as a reliable semi-quantitative tool for MOAH pre-assessment, supporting its integration into routine monitoring frameworks where rapid and broad-scale evaluation is required.

2. MATERIALS AND METHODS

2.1. CHEMICALS AND STANDARDS

All solvents used were of HPLC grade. The standard solution for external calibration included 4-ethyl-toluene CAS 99 620-14-4 purchased form Sigma Aldrich (Merck Life Science Milan-Italy) (for monoaromatics, Channel 1) and Chiron AS (Stiklestadvn, 1 N-7041 Trondheim Norway) standard mix (20 MOAH compounds, for polyaromatics, Channel 2). Naphthalene CAS 91-20-3, pyrene CAS 129-000, and coronene CAS 191-07-1 were used as retention time markers for C-fraction identification.

2.2. SAMPLE PREPARATION

Samples of well-homogenized vegetable oil or fats (0.5 g) were dissolved in isopropyl alcohol (5 ml) and mixed using vortex mixing and an ultrasonic bath when necessary. The solutions were filtered through a 0.45 µm nylon membrane into HPLC vials. Solid or highly viscous fats were preheated to ensure complete dissolution, followed by refrigerated clarification at 4°C for 5 hours before filtration.

2.3. HPLC-FLUO ANALYSIS

Analyses were performed on a Shimadzu HPLC system equipped with a C18 reverse-phase column (ReproSil 80 ODS-2, 250 mm × 4.0 mm, 3 µm). A gradient elution was used (from 60:40 to 2:98 water/acetonitrile over 50 min, held for 40 min). The flow rate was 0.4 mL/min, and injection volume was 20 µL.
Fluorescence detection was performed using dual channels:

- Channel 1: λ_{ex} = 254 nm / λ_{em} = 280 nm (monoaromatics)
- Channel 2: λ_{ex} = 254 nm / λ_{em} = 430 nm (polyaromatics)

2.4. QUANTIFICATION AND CALIBRATION

Quantification was based on external calibration using 4-ethyl-toluene and Chiron standards, with results expressed in mg/kg. C-fractions were assigned based on the retention times of naphthalene, pyrene, and coronene, though not fully aligned with JRC GC standards, as in this case the range is real for the MOAH fraction and not related to the MOSH external standard.

2.5. VALIDATION

The method was previously validated for linearity, LOQ (0.5 mg/kg), recovery (83-106%), and repeatability (CV% ~1.4%). Participation in international proficiency tests supported its accuracy and robustness [1].

3. RESULT AND DISCUSSION

A comparative analysis of MOAH quantification using HPLC-FLUO and LC-GC-FID across various edible oil matrices reveals a generally good alignment between the two methods, with matrix-dependent variations. Tables I, II, III, IV, V, VI, VII, VIII, IX, X, XI report the comparison data obtained. The complete Tables dataset including sample identification codes, matrices and analytical results is available at:
https://docs.google.com/spreadsheets/d/1OHcx8oeQThcvF7J8rpzh8UQtVZ5hI9ty/edit?usp=sharing&ouid=102913661671723803508&rtpof=true&sd=true.
For most matrices, HPLC-FLUO demonstrated satisfactory consistency with LC-GC-FID, supporting its suitability as a semi-quantitative pre-screening tool. However, discrepancies in absolute values were observed for certain matrices, likely due to differences in matrix complexity and analytical selectivity. In sunflower seed oil, which constituted the largest subset of the dataset, the two methods showed excellent agreement, confirming the robustness of HPLC-FLUO in routine monitoring of MOAH in this matrix. Conversely, grapeseed oils (both raw and refined) exhibited higher MOAH levels across both methods, with LC-GC-FID occasionally detecting slightly lower concentrations. This can be attributed to multiple contamination factors along the production chain. Firstly, grape seeds, as a by-product of winemaking, may be exposed to various sources of mineral oil contamination, including lubricants and processing aids used in harvesting and pressing.
Secondly, the extraction process - often solvent-based (e.g., using hexane) - can lead to co-extraction of aromatic hydrocarbons, especially when industrial equipment is not thoroughly maintained or cleaned.
While refining is intended to remove impurities, it may not be fully effective in eliminating MOAH, particularly when these compounds are strongly retained in the lipid matrix or are transformed during high-temperature steps such as deodorization.

Finally, contamination can also occur during storage or transport, particularly in non-dedicated or improperly cleaned containers.
These findings highlight the relevance of continuous monitoring and the importance of implementing strict controls throughout the entire production chain to minimize MOAH contamination, as well as the importance of having easy pre-screening methods available. Rice oils (crude and refined) were among the most contaminated matrices, with both techniques showing consistent trends but differing in absolute quantification. These variations could stem from matrix interferences or differential recoveries during sample preparation. This may be due to the complex and intensive processing steps required for rice bran oil production. Rice bran, in particular the raw material, is particularly prone to rapid lipid oxidation and enzymatic degradation, necessitating immediate stabilization, often involving thermal treatment or the addition of processing aids, which can be potential sources of contamination.
Moreover, solvent extraction is commonly used to maximize oil yield, increasing the risk of co-extracting mineral oil residues from equipment or solvents themselves. In general, attention must be focused on searching for solvents that are free from MOAH contaminants for use in plants.
The refining process, although effective in reducing some contaminants, may not fully eliminate aromatic hydrocarbons, particularly if they are embedded in the bran-derived matrix or introduced during high-temperature treatments.
Additional contamination may occur during storage or bulk transport if proper handling practices are not rigorously applied.
In maize seed oil and olive oils, the results were broadly comparable, though HPLC-FLUO tended to slightly underestimate MOAH compared to LC-GC-FID. Extra virgin olive oils, despite their lower expected MOAH content, showed a more pronounced discrepancy, which may be attributed to the presence of natural polyphenols that interfere with fluorescence detection. Overall, while LC-GC-FID remains the reference method for accurate quantification of MOAH, HPLC-FLUO offers a reliable and efficient alternative for pre-screening purposes, especially in high-throughput settings.
The results obtained using the RP-HPLC-FLUO technique were also found to be well-aligned with expected or reference values from the organising bodies, confirming the method's robustness and accuracy under external validation conditions (Table XII).
This alignment not only supports the method's reliability across different oil matrices but also emphasizes its reproducibility and suitability for use in collaborative settings. Despite being a semi-quantitative approach, HPLC-FLUO consistently delivered MOAH values within acceptable ranges, reinforcing its role as an effective pre-screening tool compliant with current analytical performance requirements.
Such findings provide additional confidence in the method's application for routine quality control and early risk assessment, particularly when rapid throughput and operational simplicity are essential.

4. CONCLUSIONS

The comparison between RP-HPLC-FLUO and the reference LC-GC-FID method across a wide range of vegetable oil matrices confirms the validity of the fluorometric approach as a reliable and accessible screening tool for MOAH detection. The good correlation observed between the two techniques - both in routine samples and in interlaboratory proficiency test materials - highlights the robustness, reproducibility, and practical applicability of the HPLC-FLUO method, particularly in matrices such as sunflower, grapeseed, and rice oils.
While LC-GC-FID remains the gold standard for definitive quantification, RP-HPLC-FLUO offers significant advantages in terms of simplicity, speed, and cost-effectiveness, making it ideal for high-throughput environments or for preliminary risk assessment where rapid decision-making is critical. Its ability to detect a broad range of MOAH compounds, combined with minimal sample preparation and the use of standard laboratory instrumentation, further supports its integration into monitoring programs, especially in industrial and regulatory contexts where early detection and screening are essential.
Looking ahead, future developments may focus on refining the method's selectivity to reduce matrix interferences and on expanding its application to more complex food and environmental matrices.
Overall, this study supports the continued adoption of RP-HPLC-FLUO as a valuable component of a tiered analytical strategy for MOAH surveillance.

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