UNI CT/003/GL18 Group «Oils, animal and vegetable fats and their by-products, seeds and oleagineaus fruits» MOSH and MOAH content works in progress

INNOVHUB
STAZIONI SPERIMENTALI
PER L'INDUSTRIA

innovazione e ricerca

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INNOVHUB HISTORY

- *1904 Soap Laboratory School Foundation (Soap Industries)*
- *1906 School for the fat industries in general, soaps, candles, oils, perfumes and paints Headquarters Polytechnic of Milan (Polytechnic of Milan, SIAM, Humanitarian Society, etc...)*
- *1919 Regia Experimental Station for The Oil and Fat Industries Decree 10/05/1917 SSOG*

  (Delegates of the relevant ministries and industrial associations)
- *Named Monographs: Mineral Oil Standards, Fat and Derivative Standards, Color and Paint Standards*
- *1999 Public Economic Authority under the control of the Ministry of Industry (promoting technical progress and training of operators in the sector)*
- *Subsequently control of the Ministry of Economic Development*
- *2011 Innovhub- SSI Special Agency of the Milan Chamber of Commerce – SSOG merger*
- *2017 Innovhub- SSI Special Agency of the Metropolitan Chamber of Commerce of Milan-Monza Brianza-Lodi*
- 2018 Innovhub-SSI srl wholly owned by the Metropolitan Chamber of Commerce in Milan-Monza Brianza-Lodi

NATIONAL CENTER OF INNOVATION and TECHNOLOGICAL TRANSFER

INNOVHUB MISSION

PROMOTE THE SCIENTIFIC AND TECHNOLOGICAL PROGRESS OF THE INDUSTRIAL REFERENCE SECTORS

Sectors: Agrifood sector, energy, biotechnology, nanotechnologies, bio economy, new materials, new policies and services for innovation

Activities: applied research, technical-scientific consultancy and industrial testing for manufacturing companies and provides services in the field of European design and financing for innovation, with particular attention to SMEs. Formative courses. **Participation in Standard organization**.

Standardization as base of innovation

- Link among the trasversal competences
- Manage conflicts
- Find solutions
- Deliverables faster for research project
- Project result could be a new standard
- Creates challanges and opportunities
- Networking
- Exchange of knowledge and sharing

COLLABORATIVE INNOVATION
A NEW OPPORTUNITY TO COMPETE

UNI Italian Standardization Body

Headquarters: Milan
Foundation 1921

- Private non profit association, wide participation (public and private)
- Develops volunteers technical standards in a lot of sectors (economy, industry, trade, services and society in general, excluding electrical and electrotechnical materials)
- Cooperation role between European and World Standard Organizations
- Normative culture spread
- Italy rappresentation as national Interface (Harmonization processes)

Vegetable oils

- GL 18 Oils, vegetable and animals fats and their by products, seeds and oleagineaus fruits
- GL 23 Food Authenticity (New)

- CEN/TC 275 Food analysis – Horizontal methods
- CEN/TC 307 Oilseeds, vegetable and animal fats and oils and their by-products-Methods of sampling and analysis
- CEN/TC 460 Food Authenticity

- ISO/TC 34 SC2 Oleaginous seeds and fruits and oilseed meals
- ISO/TC 34 SC11 Food products Animal and vegetable fats & oils

Mineral oils UNI works 2018-2019

- 2018 Technical paper on Mineral Oil - Agrifood Commission - UNI GL18 Oils and fats animals and vegetables and their by products, seeds and oleagineaus fruits"

All group members have shared the **CONCLUSION**

"On the base of the background material analyzed and by all experimental working group developed around this topic, the LOQs of the methods tested and available for the laboratories that mean perform the evaluation of MOSH and MOAH, are higher than the actual North European limit requested in vegetable oils".

Document distributed (SISSG) to all vegetable oils national industries as associations required

Mineral oils UNI works 2018-2019

- 2018 First Collaborative study - Agrifood Commission - UNI GL18 Oils and fats animals and vegetables and their by products, seeds and oleagineaus fruits"

Coordinators: *Dr.* Serani – Coteca, *Dr.* Di Blasi - Salov

Samples: 6 extra virgin olive oils spiked with motor oil and lubricant paste (0,0 – 0,5%)

1 refined olive oil mixed with refined pomace oilve oil

Laboratories: 10 interested, 8 results

Method: free, used for laboratory routine (MOSH and MOAH)

Scope: results comparison with different methods and observe the influence of sterenes and squalene isomers deriving from refining process

Mineral oils UNI works 2018-2019 First collaborative study

MOSH RESULTS

SAMPLES	Min-Max	Outlier	Average N=8	Standard deviation	CV R	R	Ue
	mg/kg	n	mg/kg	mg/kg	%	mg/kg	mg/kg
EVOO	2-82	1	16	10,4	64,8	36	10
EVOO 0,3% motor oil	10-41	0	25	10,6	42,7	35	9
EVOO 0,5% motor oil 0,4% lubricant paste	16-80	2	**30**	10,8	35,9	39	11
EVOO 1,5% lubricant paste	46-77	1	59	10,5	17,7	35	9
EVOO 3,5% lubricant paste	95-147	2	126	4,9	3,9	18	5
Ref. OO/Ref. Pomace OO 50/50	11-129	3	72	20,3	28,2	68	21

Mineral oils UNI works 2018-2019 First study

MOAH RESULTS

SAMPLES	Min-Max	Outlier	Average N=8	Standard deviation	CV R	R	Ue
	mg/kg	n	mg/kg	mg/kg	%	mg/kg	mg/kg
EVOO	1-15	2	2	0,8	55,8	3	1
EVOO 0,3% motor oil	1-22	1	3	1,8	70,5	6	2
EVOO 0,5% motor oil 0,4% lubricant paste	2-25	2	4	2,1	56,3	8	2
EVOO 1,5% lubricant paste	2-9	0	6	2,3	37,8	8	2
EVOO 3,5% lubricant paste	2-22	2	11	2,9	27,0	10	3
Ref. OO/Ref. Pomace OO 50/50	11-129	3	5	20,3	28,1	5	2

Mineral oils UNI works 2018-2019 Second collaborative study

- 2018 Second Collaborative study - Agrifood Commission - UNI GL18 Oils and fats animals and vegetables and their by products, seeds and oleagineaus fruits"

Coordinators: *Dr*. Serani – Coteca, Dr. Di Blasi - Salov

Samples: 5 samples of MOSH and MOAH of known content in solvent

Laboratories: 7 results

Method: no indication, that used as routine (MOSH and MOAH)

Scope: observe if without the lipidic matrix interference the results could be better

Mineral oils UNI works 2018-2019 Secodn study

MOSH RESULTS

SAMPLES	Waited content	Min-Max	Average N=7	Outlier	Standard deviation	CV R
	mg/kg	mg/kg	mg/kg	n	mg/kg	%
S0	0	0-14	0,5	1	0,58	115
S1	2	2-9	6,0	0	1,87	31
S2	130	95-140	123,6	0	17,67	14
SA1	**9**	8-11	9,4	0	1,14	12
SA2	43	25-45	44,3	0	0,96	2

Mineral oils UNI works 2018-2019 Second study

MOAH RESULTS

SAMPLES	Waited content	Min-Max	Average N=7	Outlier	Standard deviation	CV R
	mg/kg	mg/kg	mg/kg	n	mg/kg	%
S0	0	0-10	0,3	1	0,58	173
S1	0	0-10	0,3	1	0,58	173
S2	0	1-28	1,7	1	2,08	125
SA1	1	1-21	1,0	1	0,00	0
SA2	**5**	2-24	2,3	1	0,58	25

Mineral oils UNI works 2018-2019 SHARED CONCLUSIONS

- Need to develop an alternative method to *on-line*HPLC-GC-FID ISO 16995:2017
- Certified material
- Develop an off-line method (only few laboratories use it)
- Available for all laboratories, no high level instrumentation, no specialized technicians
- Able to detect, distinguish and measure the two fractions MOSH and MOAH
- Enviromental friendly
- No time consuming
- Certified material missing

Draft Project and Thesis development in collaboration with Milan University

Analytical Methods and Criticity

- ISO 11780:2015 – Oils and fats vegetables and animals raw and refined

  Determination of aliphatic hydrocarbons in vegetable oils.

  MOSH quantification content from C10 to C56

  Applicability: Content 50 mg/kg -1000 mg/kg.

- UNI EN 16995:2017- Food products based on vegetable oils and vegetable oils raw and refined

  Determination of mineral oil saturated hydrocarbons (MOSH) and mineral oil aromatic hydrocarbons (MOAH) with on-line HPLC-GC-FID

  MOSH and MOAH quantification content from C10 to C50

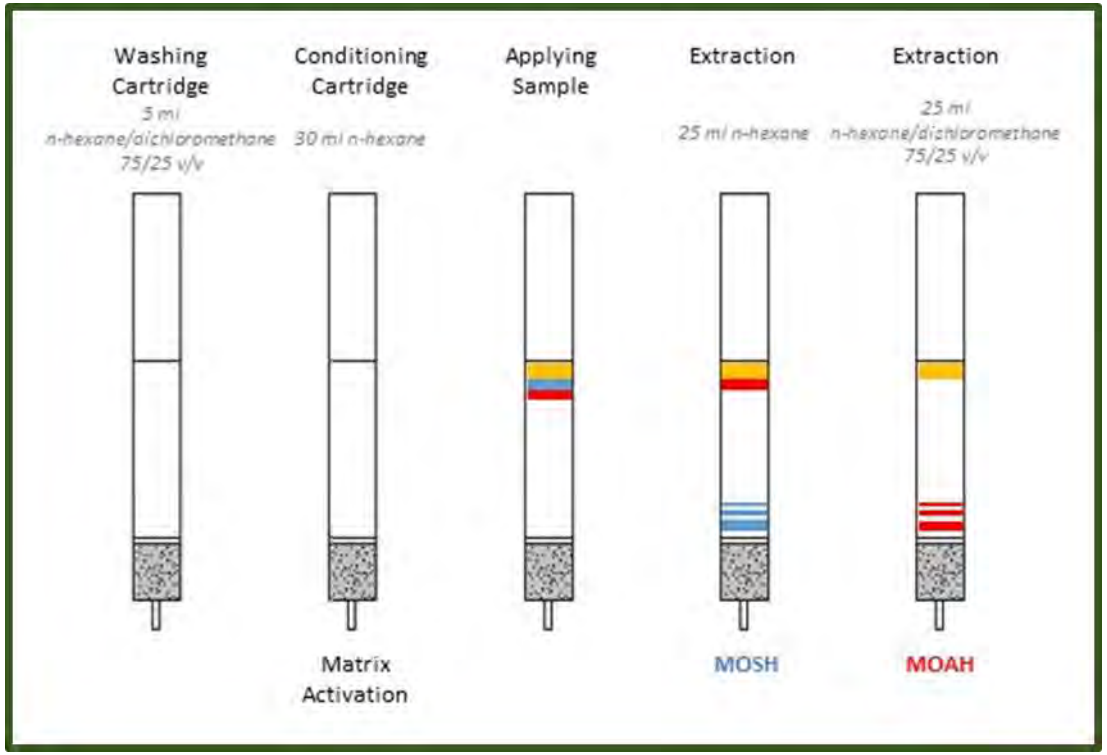
  Applicability: Content > 10 mg/kg

off-line GC-FID DRAFT Method – SAMPLE PREPARATION

MOSH and MOAH separation

- Sample: 0.5 g oil and 1 ml hexane + IS solution
- (I.S1 **H44** and H18 0.0150 mg in 100 µl of toluene)
  (I.S2 TTB and **Perylene** 0.0150 mg in 100 µl of toluene)
- E.S.: Alcani H10-H40 mix and H11, H13, CyCy, 1-MN, 2-MN
- MOSH: 25 ml of hexane
- MOAH: 25 ml of (*n*-hexane/dichloromethane) 75/25 v/v
- Fractions dried under weak nitrogen current and taken to volume 0,5 ml of isooctane and injected in a GC-FID system.
- Ratio H18/H44
- Blank for each batch of analysis

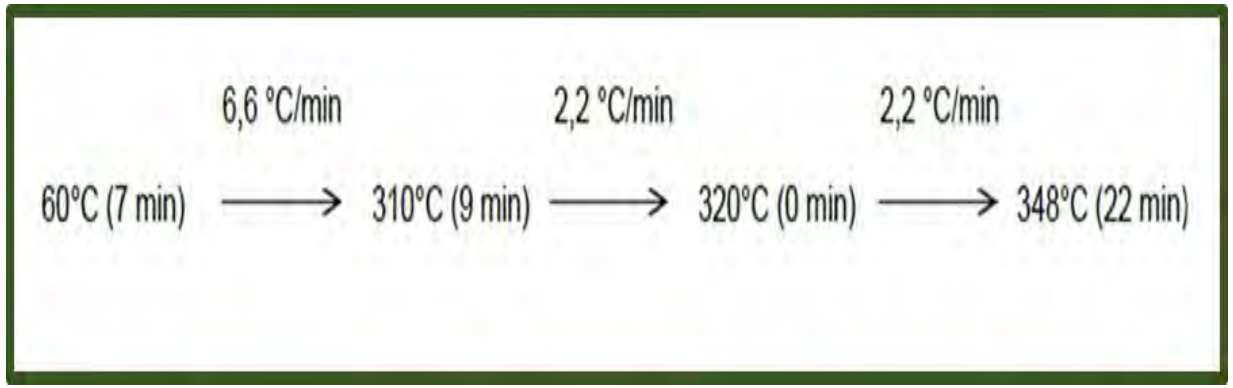
SPE glass column 15 cm * 1,5 cm and Vac Elut

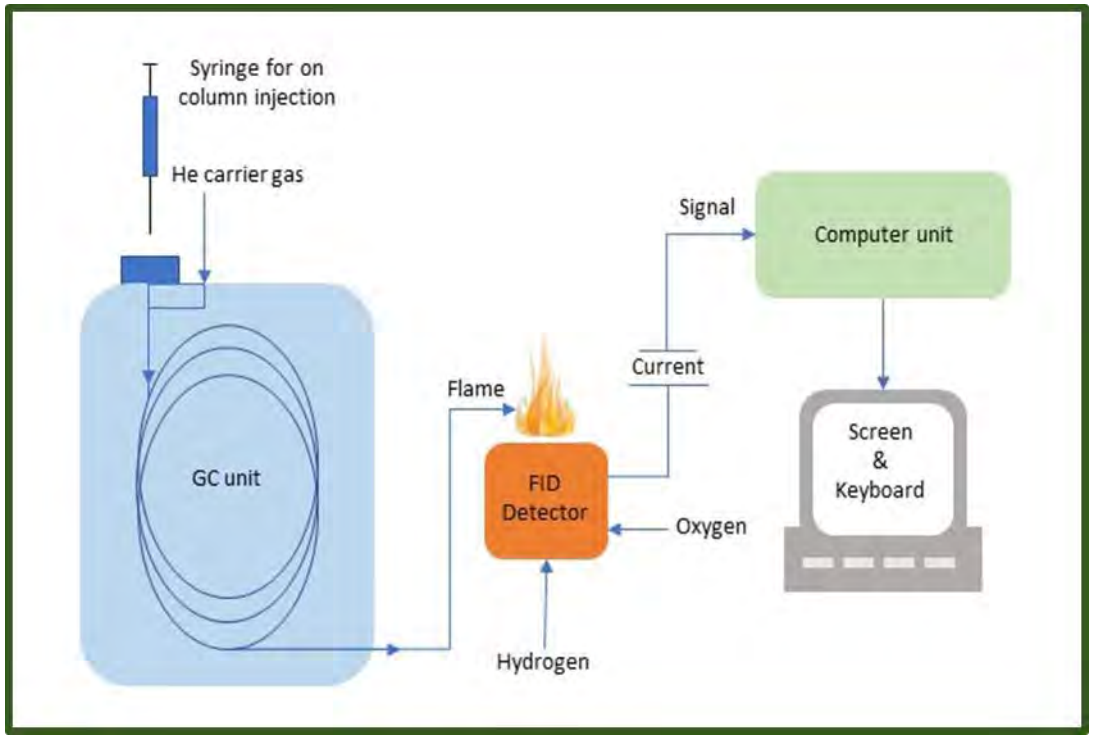


1 g silica 10% AgNO3
0.5 g di sodium sulphate anydrous
6.5 g hydrated silica 2%.

off-line GC-FID DRAFT Method – Conditions

- Column: MEGA-SE52 length 8 m; diameter 0.32 mm; thick film 0.10 µm
- Stationary phase: 5% Phenyl, 95% Methyl Polysiloxane;
- Carrier Gas: He;
- Carrier gas flow: 1.2 ml/min;
- Temperature program:





- Detector: FID

Academic Year: 2018-2019

CHROMATOGRAPHIC APPROACHES FOR THE ANALYSIS OF SATURATED AND AROMATIC MINERAL OIL HYDROCARBONS

MILAN UNIVERSITY
FACULTY OF PHARMACEUTICAL SCIENCES
Degree Course in Chemical-Toxicological Sciences and Safety of the Environment (Class L-29)

Relator: Prof. Patrizia RESTANI
Correlator: Dr. Pierangela ROVELLINI

«*Contaminanti emergenti negli oli vegetali*» Palazzo Turati – Milano 15 novembre 2019

UNI XXXX Draft method proposal

- Determinazione del contenuto di MOSH e MOAH negli oli vegetali – Metodo offline GC-FID

- INTRODUZIONE
- Si identifica come olio minerale (MOH) l'insieme degli idrocarburi di origine minerale, i quali costituiscono una miscela complessa. Essi si classificano principalmente come MOSH (idrocarburi minerali saturi comprendenti paraffine e nafteni) e MOAH (idrocarburi minerali aromatici, composti da molecole mono o poli aromatiche principalmente alchilsostituite).
- Il metodo è basato sul frazionamento di MOSH e MOAH tramite cromatografia liquida su una colonna in vetro composta da silice idratata (per eliminare eventuali composti interferenti presenti nella matrice grassa) e da silice argentata (per consentire la separazione tra MOSH e MOAH) collegata con un sistema di estrazione per SPE (Solid Phase Extraction).

- 1 – SCOPO E CAMPO APPLICAZIONE
- Il metodo descrive un procedimento per la separazione e determinazione della frazione satura (MOSH) e della frazione aromatica (MOAH) dell'olio minerale in oli vegetali di oliva e di semi, greggi e raffinati con il contemporaneo allontanamento degli interferenti dovuti alla presenza della matrice grassa dell'olio. La rivelazione e la quantificazione delle frazioni sono effettuate mediante GC-FID.

Validation process/Standardized method

OBSERVATIONS to off-line GC-FID Draft method

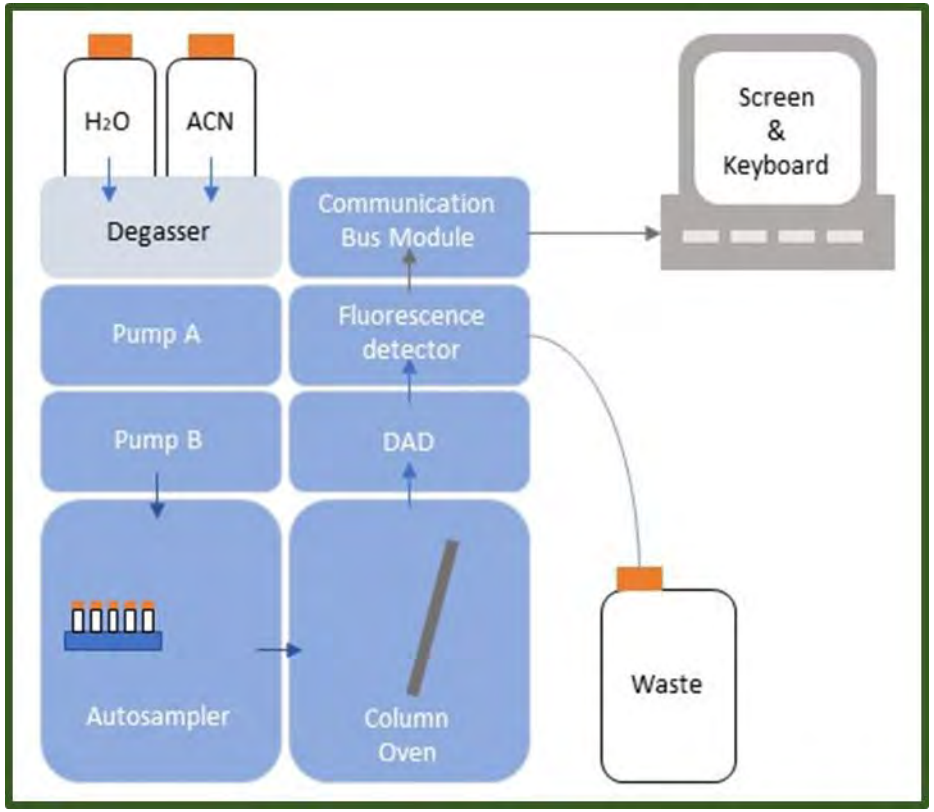
- Difficult to obtain an adeguate blank sample
- Dedicated and separated glassworks
- Evaporative process, volatile compounds partial lack (low carbon number)
- Lack of guidelinees for gaussian integration and different separation ranges
- Select the best standardized pre-purification matrix (interferences due refining process) (epoxydation, saponification, alumina column)
- Applicability > 10 mg/kg, not adeguate to the demand of the Northern European market

HPLC Fluorimetric conditions – MOAH analysis

GO WHERE YOUR IDEAS CARRY YOU

- Column: Reprosil 80 ODS-2 lenght 250 mm; diameter 4.0 mm; thick film 3.0 µm. Stationary phase: C18 (Reverse phase);
- Mobile phase: gradient
  - A: Water
  - B: Acetonitrile

Tempo (min)	A%	B%
0	60	40
50	0	100
70	0	100
71	60	40
85	60	40



Exc. 254 nm Em. 280 nm monoaromatics and alkylated
Exc. 254 nm Em. 430 nm polyaromatics and alkylated

HPLC-FLUORIMETRIC Results

- Fluorescence detector could be a good invegative tool for aromatic fraction in mineral oil more specific
- The selection of two appropriate channels of the detector could distinguish monoaromatic molecules form polyaromatic
- Sensibility at ug/kg level
- GC-FID can't discriminate monoaromatics from polyaromatics if MS is not present
- Instrumentation already present in laboratory
- No sterenes interferences, no silver silica need or acidic epoxydation
- Cheap
- Investigate MOAH and if present proceed to MOSH analysis
- Internal standard? (to be defined)
- External standard? (to be defined) Custom solution representative of investigated compounds
- MOAH GC analysis correlation

Bibliographic reference Laboratory Information Bulletin N. 4475 FDA/ORA/DFS pag. 1-39

Toxicity

MOSH

- primarily absorbed by the gut into the lymphatic system
- target organs: liver and mesenteric lymph nodes
- liver: maximum concentration after 24 h from ingestion and elimination of 85-90 % after 12 months
- patients with hepatic diseases like carcinomas, hepatites, portal fibrosis had hepatic deposits of mineral oil, the same for who consumed paraffin oil for laxative purposes
- Abdominal fat: C16-C35 centered on C23-C24
- Excretion: faecal and urinary, unmetabolized in milk
- IARC: not classifiable as carcinogenic
- No chromosomic aberrations, no reproductive toxicity

MOAH

- primarily absorbed through gastrointestinal tract
- target organs: spleen, liver kidney and brain
- liver: maximum concentration after 1-2 h from ingestion . Able to cross placenta.
- Excretion: faecal and urinary, unmetabolized in milk
- IARC: exposure to mineral oils containing aromatic compounds is linked to the development of squamous-cell cancers of skin and scrotum
- PAHs between 3 and 7 rings are recognised mutagenic and genotoxic
- Maternal toxicity and foetal toxicity

Contact

Innovation is our tradition

Thanks for your attention

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