



Characterization of olive oils in accordance with EEC regulation No. 2568/91 and subsequent amendments. Fully automated analysis of sample preparation.

PART 1:

Methyl / Ethyl Esters and Waxes

Current EU legislation provides for objective criteria aimed at classifying the various types of olive oils (virgin, clear, refined, etc.).

These criteria are explained by the EEC regulation No. 2568/91, with reference to the latest revision of 20/10/2019.

Assigning each oil to the correct class it belongs to is of fundamental importance for:

- guarantee the commercialization of olive oils that meet the characteristics declared on the label
- avoid potential adulteration, even of a malicious nature
- ultimately protect the health and interests of the final consumer:

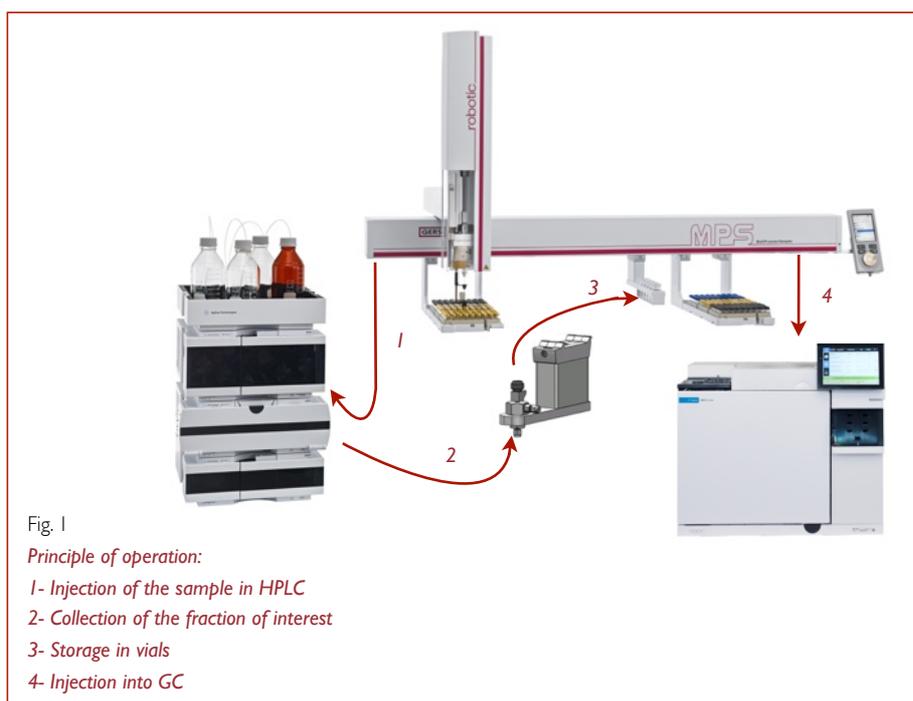
Annexes II ÷ XX of the regulation define the analytical methods relating to the quantification of the parameters of interest; many of these involve laborious sample preparation, large quantities of solvents and consumables, as well as the use of qualified operators for a long time.



SRA Instruments, in collaboration with the Centro Analisi Biochimiche, has conducted an in-depth study of the methods in use, coming to offer a series of analytical solutions capable of fully automating the sample-prep phases, with consequent savings in terms of time, solvent and materials.

The use of robotic stations also allows you to greatly limit the possibility of incurring random errors, as well as keeping the process under control by inserting a large number of QCs within a batch; in this way, not only high productivity is guaranteed, but also an extremely reliable final data in terms of precision and accuracy.

The robotic station, object of this application note, is able to fully automate the sample preparation process and the subsequent analysis aimed at determining the content of alkylesters and waxes.



Method automation

The guiding principle of the solution consists in the elimination of off-line preparative techniques (LC, TLC, LLE), replacing them with an automated separation of the fractions of interest via HPLC, as shown in Figure 1.

Specifically, the careful optimization of the chromatographic parameters makes it possible to make the elution times of the various classes (methyl / ethyl esters and waxes, in this case) extremely repeatable. The withdrawal of this fraction and the subsequent injection in LVI-COC-FID mode immediately provides the analytical data, limiting the operator's intervention to the simple start of the analysis sequence.

Effectiveness of automation

To highlight the increase in productivity, with the simultaneous drastic reduction of solvent and necessary consumables, in tab. I shows the comparison between the traditional method (Annex XX to EEC regulation No. 2568/91) and the one implemented on the proposed platform.

Complete analysis of alkylesters & waxes	Traditional method	HPLC / GC automation
preparation by the operator	Column preparation for LC Sample loading and elution Evaporation to dryness Recovery with solvent	Dilution of the initial sample
volume of solvent per sample	~ 300ml (plus 15g pre-conditioned silica)	~ 20 ml
volume of solvent for a batch of 10 samples	~ 3000ml (plus 150g of pre-conditioned silica)	~ 200 ml
time required to process a single sample	~ 2 h	<1 h
time required to process a batch of 10 samples	~ 12 h, of which ~ 4 needed for batch preparation	~ 8 h of which ~ 30 minutes required for batch preparation

A further advantage of the system is the possibility to selectively collect one of the two fractions in order to:

- standardize the method to the provisions of Annex IV to EEC Regulation No. 2568/91, with similar advantages in terms of time and consumption of solvents and silica (collection of the "wax" fraction only)
- eliminate the problem related to the presence of high concentrations of matrix interferents, which could prevent the correct dosage of the alkyl esters (collection of the "alkylester" fraction only).

In the case of the analysis of alkylesters alone, a further reduction of analysis times is obtained up to less than 30 minutes for the single sample (approximately 6 hours for a batch of 10).

Analytical performance

Once the undoubted advantages related to the automation of the preparation process, it is equally important to verify that the final data is characterized by a level of analytical reliability equal to or greater than that guaranteed by the traditional method. About this, a series of experimental tests were carried out using a virgin reference oil as control sample, whose content in alkylesters and waxes is certified by the interlaboratory circuit of the Chamber of Commerce of Rome - Special Samer Ring Test. N. 62 (RT62). Different aliquots of the same sample, interspersed with process blanks, were processed by the platform automatically and without any operator supervision. The standard chromatogram (in blue), compared with that reported in the EEC regulation (in black), is shown in figure 2.

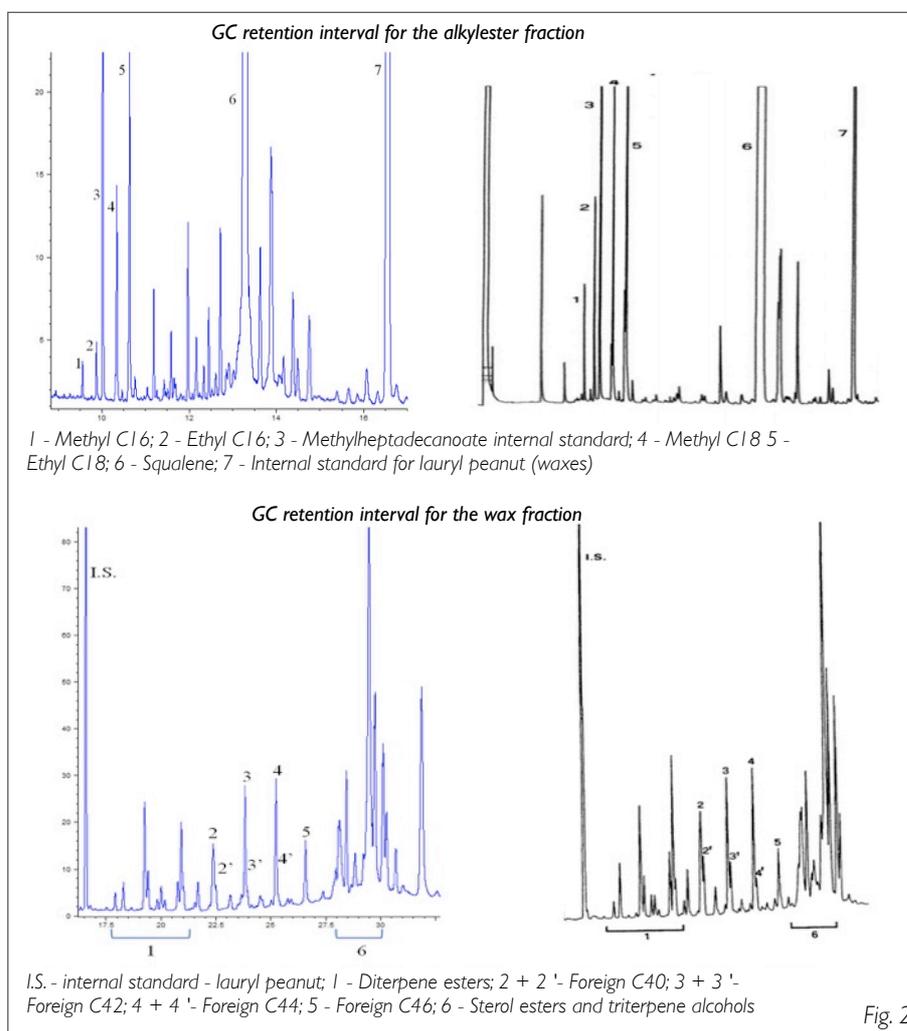


Fig. 2

The result of the validation batch on a series of 10 repetitions, shown below, highlights the absolute reliability of the data in terms of accuracy and precision:

WAXES validation			Alkylesters validation		
#	mg/kg (sum)		#	mg/kg FAEE (sum)	
1	225.8	Average 220.5	1	36.6	Average 35.4
2	216.9		2	35.6	
3	215.2	St. dev. 5.1	3	36.0	St. dev. 0.53
4	215.5		4	35.5	
5	227.3	Reference value (as per RT62) Waxes = 221.0	5	35.0	Reference value (as per RT62) FAEE = 36.2
6	215.1		6	35.2	
7	224.2	Bias% 0.25%	7	35.0	Bias% 2.18%
8	224.7		8	35.0	
9	215.8	CV _r % 2.32%	9	35.2	CV _r % 1.51%
10	224.0		10	35.0	

A further strength of the system is its long-term reliability.

Figure 3 shows the overlay of two control analyzes, conducted on the reference used for the validation of the system, obtained two months apart. More than 1000 chromatographic runs were performed between analyzes (real samples, QCs, blank).

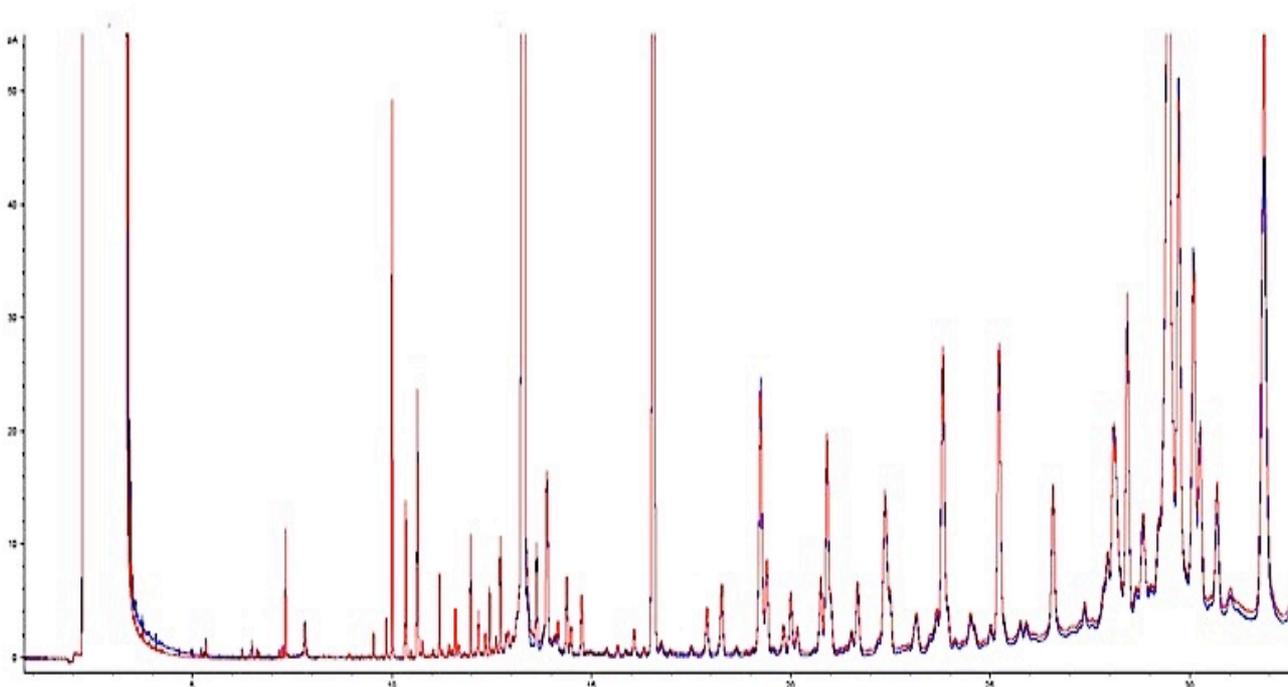


Fig. 3

Conclusions

The proposed solution allows to fully automate the sample prep procedure, with consequent reduction of costs per sample in terms of lower consumption of solvent and accessory materials.

Analysis times are significantly reduced, almost completely eliminating operator intervention.

In addition, the fact of working automatically drastically lowers the incidence of random errors, ensuring long-term precision, accuracy and robustness of the measurements.

The proposed preparatory station is part of a wider range of solutions, developed by SRA Instruments in collaboration with Gerstel GmbH and Biochemical Analysis Center Sas, aimed at automating specific applications such as:

- INANOIL series analyzers: Characterization of olive oils in accordance with EEC regulation No. 2568/91. Fully automated analysis of sample preparation
 - ♦ Part 2: Sterols and alcohols
 - ♦ Part 3: Stigmastadieni
- MOSH / MOAH analysis in accordance with the DIN EN 16995: 2017-08 method, including AIOX purification and epoxidation. <https://www.srainstruments.com/s/mosh-moah-gerstel-sample-prep-solution/>
- determination of 2 & 3 MCPD and GE according to the official AOCS Cd 29 (a & b & c) -13 methods. <https://www.srainstruments.com/s/determination-of-3-mcpd-and-glycidol-in-edible-oils-by-gc-ms/>

Similar systems are also being studied / validated in able to automate:

- the analysis of additional parameters included in the EEC regulation No. 2568/91
- online saponification of olive oil
- the determination of Polycyclic Aromatic Hydrocarbons (IPA) pursuant to EC regulation 1881/2006 and subsequent amendments.



Courtesy of Dr. Dott. Carmine Ventre, Centro Analisi Biochimiche Sas - Rizziconi (RC)