SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

INANOIL Solution

Characterization of olive oils in accordance with EEC regulation No. 2104-2105/2022. Fully automated analysis of sample preparation.

Introduction

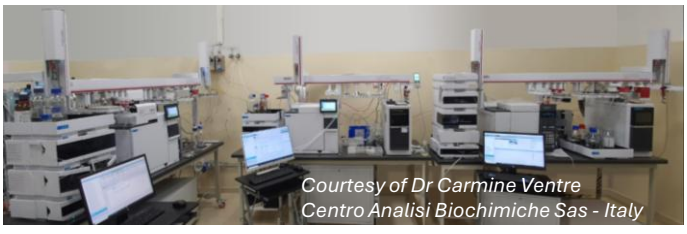
Current EU legislation provides for objective criteria aimed at classifying the various types of olive oils (virgin, clear, refined, etc.).

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 complying with 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, the use of qualified operators for a long time.



Courtesy of Dr Carmine Ventre
Centro Analisi Biochimiche Sas - Italy

SRA Instruments, in collaboration with the Centro Analisi Biochimiche Sas, has conducted an in-depth study of the methods in use, coming to offer the market **a series of analytical solutions based on the GERSTEL platform, able to fully automate the sample-prep phases, resulting in savings in terms of time, solvent and materials**.

The robotic stations are able to fully automate the sample preparation process and subsequent analyzes aimed at determining, respectively, the content of:

1. **alkyl esters and waxes**
2. **sterols, triterpene di-alcohols and aliphatic alcohols**
3. **stigmastadienes**.

The platforms 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.

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. Specifically, the accurate optimization of the chromatographic parameters makes it possible to make the elution times of the various classes.

Alkylesters and waxes: full automation of the sample preparation and analysis process

Off-line preparative techniques (LC, LLE) are replaced with an automated separation of the fractions of interest via HPLC (Figure 1). The careful optimization of the chromatographic parameters makes it possible to make the elution times of methyl / ethyl esters and waxes 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.

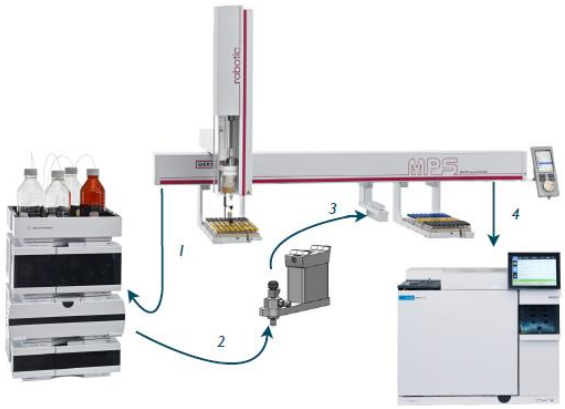


Fig. 1
Principle of operation:
1. *Injection of the sample in HPLC*
2. *Collection of the fraction of interest*
3. *Storage in vials*
4. *Injection into GC*

Effectiveness of automation

To highlight the increase in productivity, with the simultaneous drastic reduction of solvent and necessary consumables, the table alongside shows the comparison between the traditional method and the one implemented on the INANOIL platform.

Another advantage is that the system allows for the selective collection of one of the two fractions to:

- standardize the method by current legislation, 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 alkyl esters (collection of the “alkylester” fraction only).

In the case of the analysis of alkylesters alone, a further reduction in analysis times is obtained (less than 30 min. for the single sample).

Analytical performance

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 Bari Chamber of Commerce - Special Samer Ring Test. N. 62 (RT62).

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

Waxes validation	
Average 3.70	St.: Dev..: 5.1
Reference value (as per R T62) waxes= 221.0 mg/Kg	
BIAS = 0.25 %	CV_r%= 2.32%

Alkylesters validation	
Average: 3.70	St.: Dev.: 0.5
Reference value (as per R T62) FAEE = 36.2 mg/Kg	
BIAS = 2.18%	CV_r% = 1.51%

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
Solvent per sample volume	300 ml (plus 15g preconditioned silica)	~ 20 ml
10 samples batch solvent volume	3000 ml (plus 150g of preconditioned silica)	~ 200 ml
Single sample process time	~ 2 h	<1 h
10 samples batch process time	12 h, of which ~ 4 needed for batch preparation	~ 8 h of which ~ 30 minutes for batch preparation

Sterols and alcohols: full automation of the sample preparation and analysis process

Off-line preparative techniques (LC, TLC, LLE), are replaced with an automated separation of the fractions of interest via HPLC (Figure 2).

Specifically, the accurate optimization of the chromatographic parameters makes it possible to make the elution times of the various classes (aliphatic alcohols and sterols / triterpene dialcohols, in this case). The versatility of the MPS RoboticPRO platforms allows you to extend the automation of the workflow by including the necessary steps of evaporation to dryness and recovery with derivatizer. The subsequent injection in SSL-FID mode immediately provides the analytical data, limiting the operator's intervention to the simple start of the analysis sequence.

Effectiveness of automation

The automation of the sample-prep starts from the unsaponifiable fraction prepared in accordance with the official method (see Annex XIX Part 1). This fraction is placed on the sampler and processed automatically, completely avoiding the separation procedure via TLC. To highlight the increase in productivity, with a simultaneous drastic reduction in solvent and necessary consumables, the table below shows the comparison between the traditional method (Att. XIX to EEC regulation No. 2568/91) and the one implemented on the INANOIL platform. The SRA solution, with an HPLC separation conducted in less than 5 minutes, automates the entire process up to the GC injection. The automatic management of analytical times also allows an overlap of GC and HPLC runs; in fact, the time required for the analysis is reduced to just the GC run.

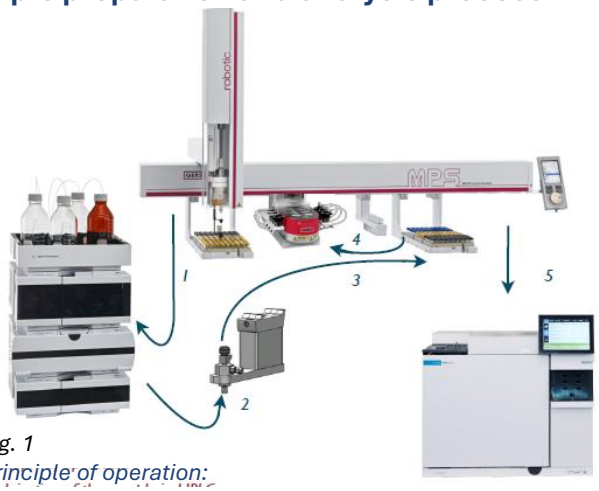


Fig. 1
Principle of operation:
1. *Injection of the sample in HPLC*
2. *Collection of the fraction of interest*
3. *Storage in vials*
4. *Dry evaporation and derivatization*
5. *Injection into GC*

Analytical performance

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 Bari Chamber of Commerce - Special Samer Ring Test. N. 62 (RT62).

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

Alifatics alcohols validation	
Average: 3.70	St.: Dev.: 4.0
Reference value (as per R T62) alcohols = 258.7 mg/Kg	
BIAS = 1.46%	CV_r% = 1.53%

Sterols and e triterpenic dialcohols validation	
Average 3.70	St.: Dev.: 25
Reference value (as per R T62) Sterols = 1447 mg/Kg	
BIAS = 1.73%	CV_r%= 1.74%

Complete analysis of sterols and alcohols	Traditional method	HPLC/GC automation
Preparation by the operator	TLC separation of the unsaponifiable fraction Evaporation to dryness Recovery with derivatization	Positioning of the unsaponifiable fraction on the autosampler
Solvent per sample volume	> 1000 ml, in relation to the size of the developing chamber, TLC plate	~ 15 ml
10 samples batch solvent volume	~3000 ml, 10 TLC plates	~ 150 ml
Single sample process time	~ 3 h	<1,5 h
10 samples batch process time	~10 hours, of which ~4 hours are required for batch preparation	7 h, of which ~ 30 minutes required for batch preparation

Stigmastadienes: full automation of the sample preparation and analysis process

Off-line preparative techniques (LC, LLE) are replaced with an automated separation of the fractions of interest via HPLC, as shown in Figure 3.

This analytical approach also allows to eliminate the saponification step.

Specifically, the accurate optimization of the chromatographic parameters makes it possible to make the elution times of the fraction containing the stigmastadienes extremely repeatable, eliminating the interference constituted by squalene. The withdrawal of this fraction and the subsequent injection in MMIFID 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, the table below shows the comparison between the traditional method (Annex XVII to the EEC regulation No. 2568/91) and the one implemented on the platform INANOIL.

The SRA solution, with an HPLC separation conducted in less than 5 minutes, automates the entire process up to the GC injection. The automatic management of analytical times also allows an overlap of GC and HPLC runs; in fact, the time required for the analysis is reduced to just the GC run.

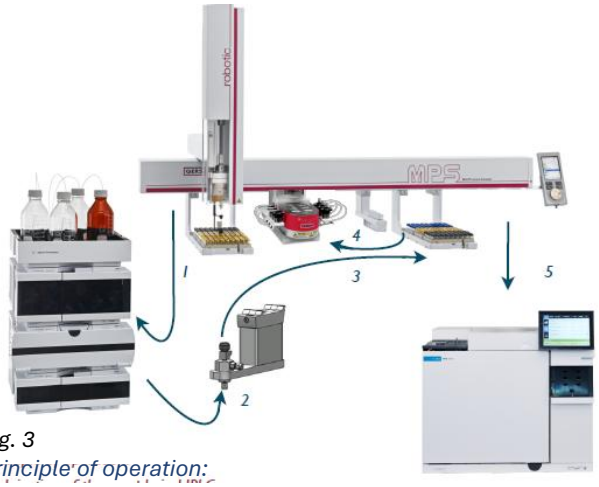


Fig. 3
Principle of operation:
1. *Injection of the sample in HPLC*
2. *Collection of the fraction of interest*
3. *Storage in vials*
4. *Evaporation to dryness and recovery with solvent*
5. *Injection into GC*

Analytical performance

A series of experimental tests were carried out using a virgin reference oil as control sample, whose concentration of stigmastadienes is certified by the interlaboratory circuit of the Rome Chamber of Commerce - Special Samer Ring Test Company. No. 62 (RT62). The result of the validation batch on a series of 10 repetitions, highlights the absolute reliability of the data in terms of accuracy and precision.

Stigma RT62 validation	
Average: 3.70	St.: Dev.: 0.04
Reference value (as per R T62) Stigma = 3.70 mg/Kg	
BIAS = 1.66%	CV_r% = 1.16%

Stigma cut-off validation	
Average 3.70	St.: Dev.: 0.004
Reference value (as per R T62 dil 1/65) Stigma = 0.057 mg/Kg	
BIAS = 6.03%	CV_r%= 6.48%

Complete analysis of sterols and alcohols	Traditional method	HPLC/GC automation
Preparation by the operator	Saponification Column preparation for LC Sample loading and elution Evaporation to dryness Recovery with solvent	Dilution of the initial sample
Solvent per sample volume	> 400 ml (plus 15 g of preconditioned silica)	<20 ml
10 samples batch solvent volume	> 4000 ml (plus 150 g of preconditioned silica)	~ 200 ml
Single sample process time	~ 3 h	<1 h
10 samples batch process time	~ 15 h, of which ~ 10 needed for batch preparation	<10 h of which ~ 30 minutes required for batch preparation

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