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

PART 3:

Stigmastadiens

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, 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, high productivity and extremely reliable final data in terms of precision and accuracy are guaranteed.

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 stigmastadienes content.

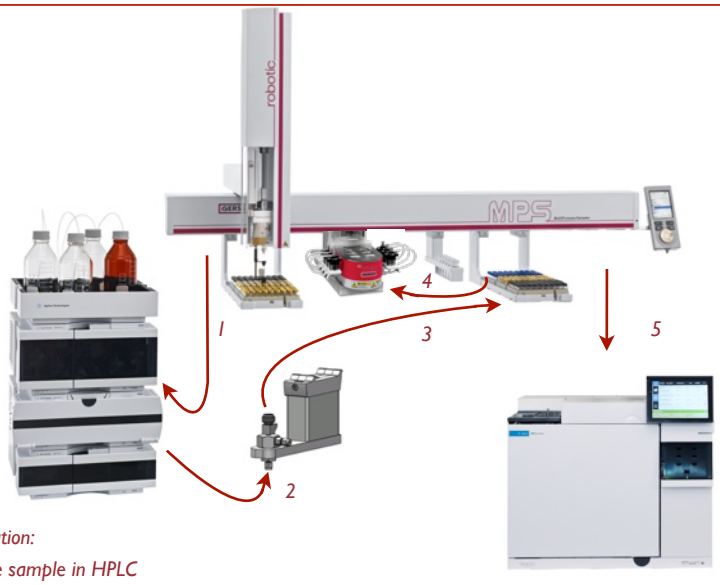


Fig. 1
Principle of operation:
1- Injection of the sample in HPLC
2- Collection of the fraction of interest
3- Storage in vials
4- Evaporation to dryness and recovery with solvent
5- Injection into GC

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; **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 MMI-FID mode immediately provide 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 table 1 is shown the comparison between the traditional method (Annex XVII to the EEC regulation No. 2568/91) and the one implemented on the proposed platform.

Complete stigmastadiens analysis	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
volume of solvent per sample	> 400 ml (plus 15 g of pre-conditioned silica)	< 20 ml
volume of solvent for a batch of 10 samples	> 4000 ml (plus 150 g of pre-conditioned silica)	~ 200 ml
time required to process a single sample	~ 3 h	~ 1 h
time required to process a batch of 10 samples	~ 15 h, of which ~ 10 needed for batch preparation	<10 h of which ~ 30 minutes required for batch preparation

Table 1

Analytical performance

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

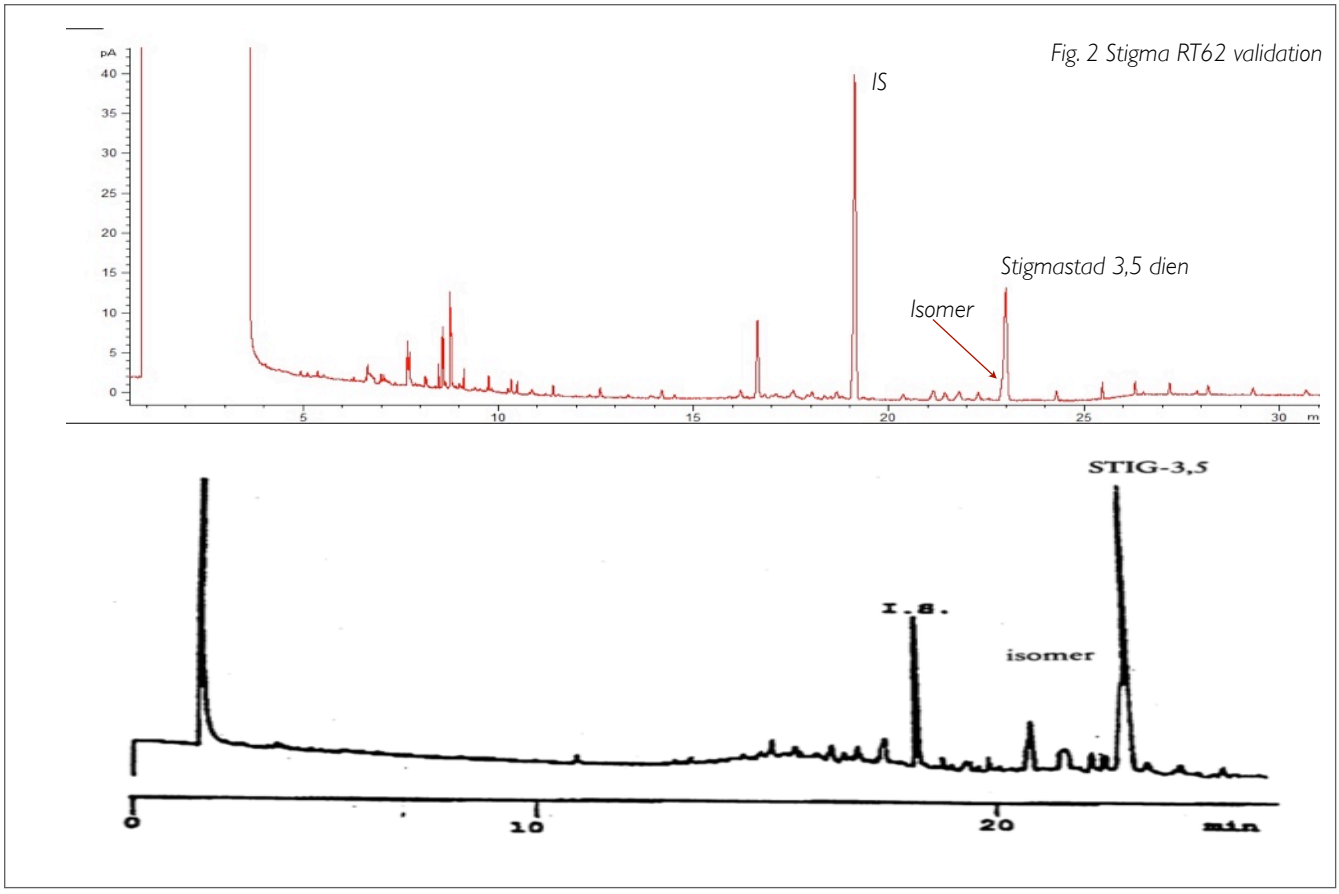


Fig. 2 Stigma RT62 validation

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, as shown in the table "Validation Stigma RT62" in fig. 3.
In order to further validate the method at the cut-off value of 50 ppm, the reference RT62 was diluted 1/65 with EVO oil, obtaining a concentration just above the expected limit of 0.05 mg / kg; the relative values are shown in the table "Stigma cut-off validation":

Stigma RT62 validation

#	mg/kg stigma
1	3.68
2	3.78
3	3.82
4	3.78
5	3.77
6	3.81
7	3.78
8	3.75
9	3.78
10	3.70

Average	St.dev.
3.70	0.04
Reference value (as per RT62) stigma = 3.70	
Bias%	CV_r%
1.66%	1.16%

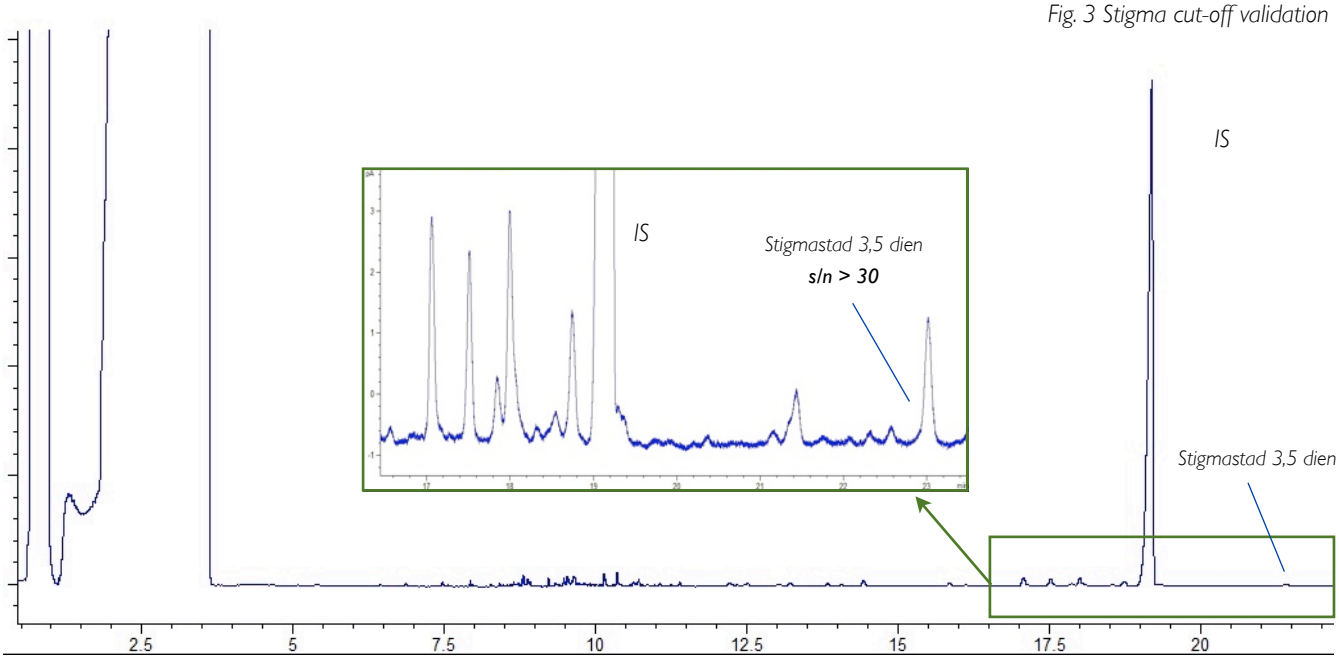
Stigma cut-off validation

#	mg/kg stigma
1	0.061
2	0.068
3	0.064
4	0.059
5	0.061
6	0.055
7	0.063
8	0.064
9	0.057
10	0.059

Average	St.dev.
0.061	0.004
Reference value (as per RT62 dil 1/65) stigma = 0.057	
Bias%	CV_r%
6.03%	6.48%

Thanks to the high efficiency of HPLC separation, the final GC trace shows, at the cut-off value, a s/n ratio higher than 30 for the analyte of interest.

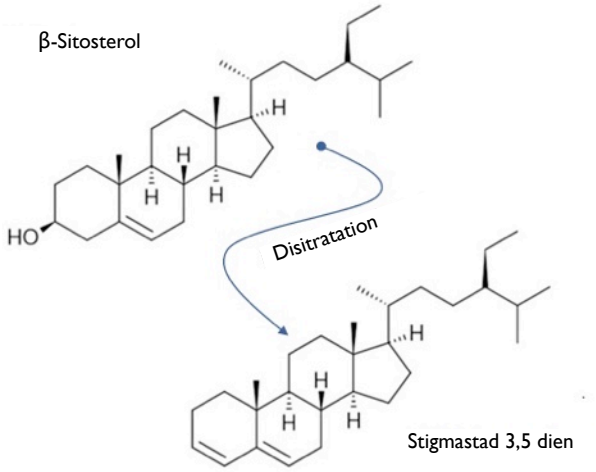
Fig. 3 Stigma cut-off validation



The aspect linked to the absence of saponification has fundamental importance: in addition to greatly reducing the preparation time, this allows a further reduction of analysis costs; costs already reduced by the saving of over 90% of the solvent necessary for the following steps, completely replaced by HPLC separation.

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 drastically reduced, almost completely eliminating operator intervention. In addition, the very fact of working automatically limits the incidence of random errors enormously, 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:

- haracterization of olive oils in accordance with EEC regulation No. 2568/91: analysis with exhaustive automation of sample preparation:
  - Part 1: Methyl / ethyl esters and waxes
  - Part 2: Sterols and alcohols
- MOSH / MOAH analysis in accordance with the DIN EN 16995: 2017-08 method, including AlOX 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 capable of automating:

- 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)

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