

EXTENDED AUTOMATION OF OLIVE OIL ANALYSIS ACCORDING TO EU REGULATION 2022/2104 AND 2022/2105



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INTRODUCTION & BACKGROUND

The regulatory classification of olive oils, mandatory at the EU level, requires the evaluation of several parameters to determine the specific quality grade. Many of these analyses involve time-consuming sample preparation steps, characterized by high solvent consumption, intensive manual labor, and significant waste of consumables. The aim of this study was to develop, validate, and assess the robustness of automated analytical procedures based on sustainable analytical chemistry, specifically designed for the determination of alkyl esters, waxes, sterols, alcohols, and stigmastadienes.

TECHNICAL SOLUTION: SRA INANOIL AUTOMATION

The innovative INANOIL platform totally changes laboratory workflows from manual sample manipulation to an advanced HPLC/GC online hyphenation technique. This approach completely revolutionizes the perspective of traditional methods:

STANDARD METHODS	INANOIL METHODS
Alkylesters, Waxes, Stigmastadienes	Alkylesters, Waxes, Stigmastadienes
Initial weighing	Initial weighing
Saponification (as needed)	Completely automated process via HPLC fractionation → GC Analysis
LC fractionation	
Evaporation	
GC injection	
Sterols, Alcohols	Sterols, Alcohols
Initial weighing	Initial weighing
Saponification	Completely automated process via Saponification → HPLC fractionation → Derivatization → GC Analysis
Clean-up	
Evaporation	
Manual TLC fractionation	
Evaporation	
Derivatization	
GC injection	

SUSTAINABLE ANALYTICAL CHEMISTRY

By adopting the online HPLC/GC configuration, laboratories eliminate macro-scale cleanup and isolation steps. The table below outlines the resource savings achieved per batch of 10 analyses:

Analytical Parameter	Standard Official Methods	SRA INANOIL Methods
Solvents for Preparative Step	~ 10 liters	~ 0.5 liters
Solvents for Cleanup/Extraction	~ 5 liters	< 0.5 liters
Activated Silica Gel	300 g	Not Needed (Completely Eliminated)
TLC Silica Sheets	10 sheets	Not Needed (Completely Eliminated)

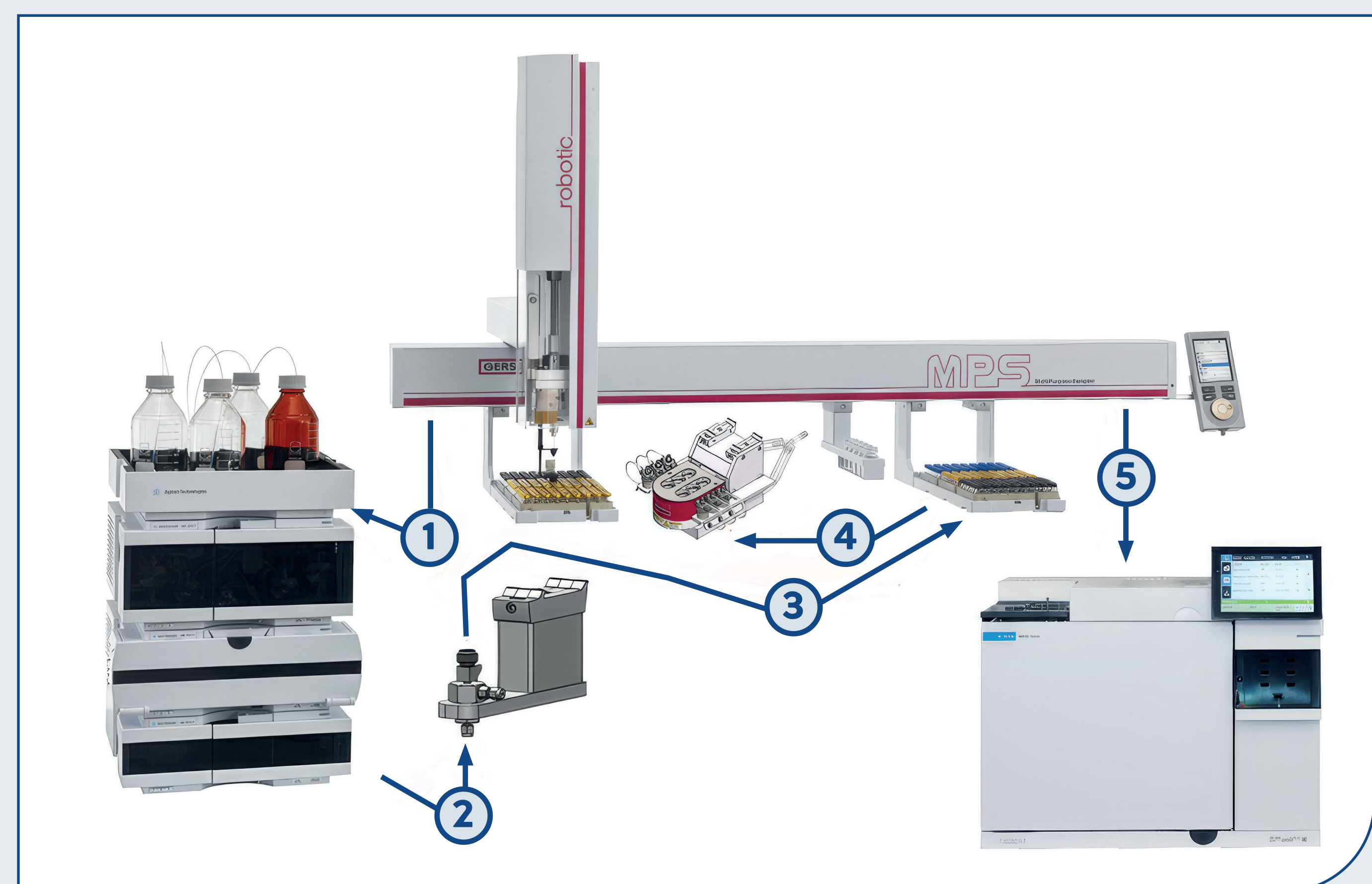
More than 90% total organic solvent saved (~14 liters reduction per 10-sample batch), completely avoiding the exposure of operators to toxic vapors and eliminating solid chemical waste generation.

ACKNOWLEDGEMENTS

- Dr. Carmine Ventre - Centro Analisi Biochimiche Sas-Italy
- Dr.ssa Rita Sechi - Umbria Olii International S.p.A.-Italy
- Dr.ssa Giulia Casano - Cavanna Olii Snc-Italy



SRA INANOIL PLATFORMS



SRA INANOIL platforms are based on HPLC/GC. Principle:

- 1 HPLC injection (whole sample or saponified)
- 2 Fraction(s) with collection
- 3 Vial storage
- 4 Further automated sample treatments, if needed
- 5 Final GC injection

Fully automated on-line analytical workflow driven by Gerstel robotics, where advanced sample treatments (including saponification, evaporation and derivatization) are completely integrated with the online HPLC/GC configuration.

METHOD VALIDATION

Method robustness and accuracy were critically verified through a rigorous internal validation process modeled after the official Ring Test «RT62» (organized by the Camera di Commercio di Roma), a particular blend of extra virgin olive oil (EVOO) and refined oil. Several 10 sample batches were tested via fully automated workflow. For each of them, statistical indicators (Average, CVr%, and BIAS%), were calculated as below:

Compound Class	Reference Value	Experimental Avg	Repeatability (CVr%)	Accuracy (BIAS%)
Waxes (mg/kg)	221.0	220.5	2.31 %	0.22 %
FAEE (mg/kg)	36.3	35.5	1.46 %	2.18 %
Sterols (mg/kg)	1447.0	1422.0	1.74 %	1.73 %
Stigmastadienes (QC)	3.70	3.76	1.16 %	1.66 %
Stigmastadienes	0.057	0.061	6.03 %	6.48 %

CONCLUSIONS

- **Complete Workflow Automation:** Enables a fully automated, operator-free analytical workflow from initial vial positioning to final report.
- **Uncompromised Data Quality:** The platform maintains extreme precision and accuracy: CVr% and BIAS% in accordance with strict regulatory acceptance criteria.
- **Environmental & Safety Compliance:** A true green lab solution showing a massive reduction (>90%) in solvent waste and total elimination of traditional disposable labware.
- **Regulatory Readiness:** Guarantees seamless conformity with European Regulations 2022/2104 and 2022/2105 for routine oil screening.