SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

INANOIL Analyzers

Automated characterization of olive oils

SISSG
SOCIETÀ ITALIANA PER LO STUDIO DELLE SOSTANZE GRASSE

Congresso SISSG 2022 "OLI E GRASSI ALIMENTARI: INNOVAZIONE E SOSTENIBILITA' NELLA PRODUZIONE E NEL CONTROLLO"

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June, 17th 2022 - Perugia

SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

INANOIL Analyzers

COMMISSION REGULATION (EEC) No 2568/91

of 11 July 1991

on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis

(OJ L 248, 5.9.1991, p. 1)

SRA INSTRUMENTS
ANALYTICAL SOLUTIONS

Analizzatori INANOIL

SOLUTION 1:

Alkyl-esters and Waxes

SOLUTION 2:

Sterols and Alcohols

SOLUTION 3:

Stigmastadienes

Further automations

MOSH/MOAH (DIN EN 16995:2017-08)

Including on-line Aloxidation and Epoxidation

2/3 MCPD e GEs

AOCS Cd 29a/b/c-13

Reference Regulation

COMMISSION REGULATION (EEC) No 2568/91

of 11 July 1991

on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis

(OJ L 248, 5.9.1991, p. 1)

Change of perspective

The methods listed in regulation often involve:

- Large amount of solvents
- Need of skilled lab technicians for several hours
- Use of large quantities of consumables and glassware
- Laborious sample preparation
- Predisposition to random errors

Automated sample-prep and analysis via HPLC/GC

- Drastic reduction in volume of solvents to be used
- The need to dedicate operators limited to a few minutes
- Almost no use of consumables and glassware
- Sample preparation substantially limited to an initial dilution
- Elimination of random errors

Traditional and INANOIL workflow comparison – Alkylesters, Waxes, Stigmastadienes

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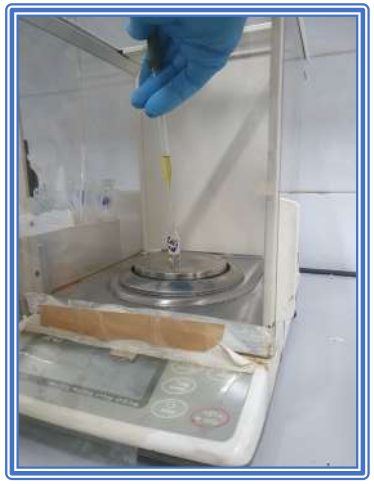
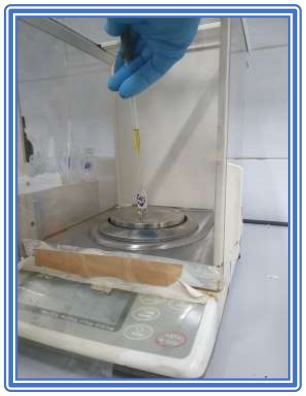
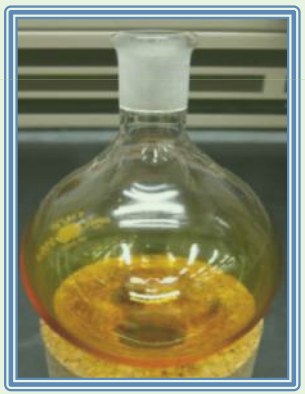
ANALYTICAL SOLUTIONS



Saponification

Traditional and INANOIL workflow comparison – Sterols, Alcohols





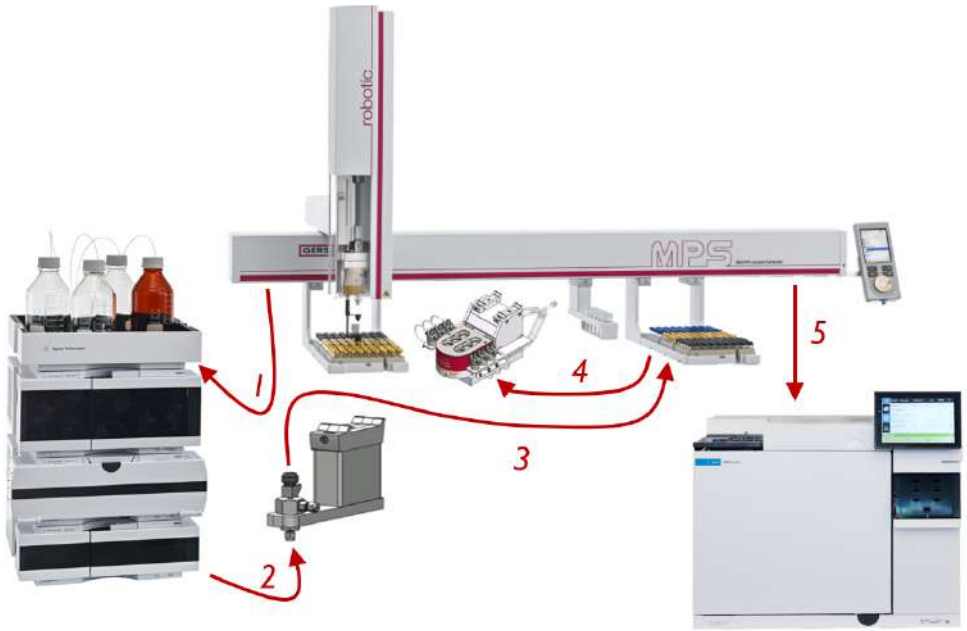
Saponification

INANOIL automated platforms

Change of perspective

Traditional methods

HPLC/GC hyphenation



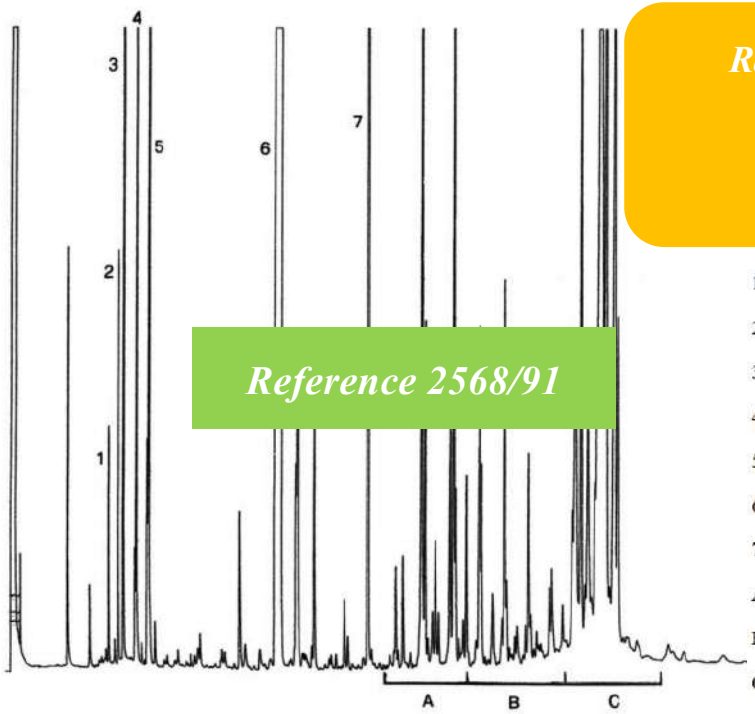
Principle

1 – HPLC injection (whole sample or saponified)
2 – Fraction(s) withdrawal
3 – Vial storing
4 – Further automated sample treatments
5 – Final GC injection

Optimisation results – Alkylesters and Waxes

ANNEX XX

Method for the determination of the content of waxes, fatty acid methyl esters and fatty acid ethyl esters by capillary gas chromatography

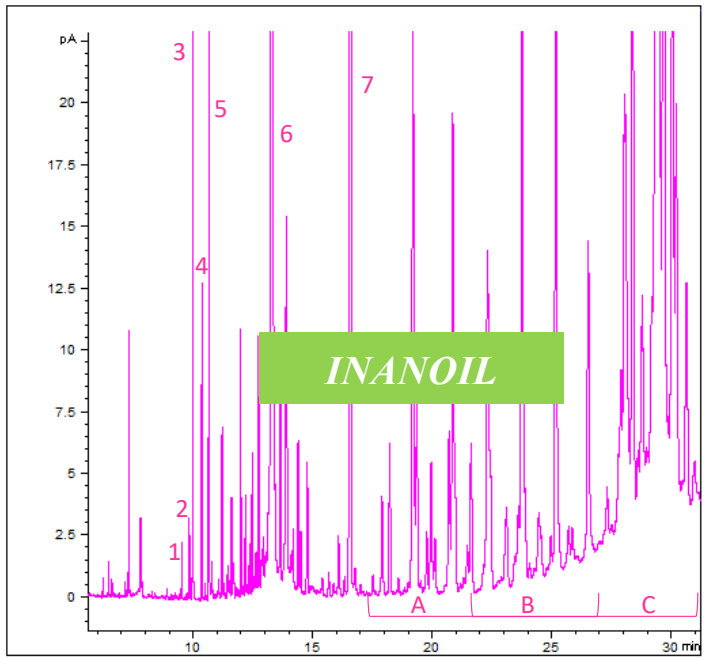


Reference 2568/91

Reference cromatograms comparison

FAME & FAEE

1 – Methyl C_{16}
2 – Ethyl C_{16}
3 – Methyl heptadecanoate I.S.
4 – Methyl C_{18}
5 – Ethyl C_{18}
6 – Squalene
7 – Lauryl arachidate I.S.
A – Diterpenic esters
B – Waxes
C – Sterol esters and triterpenic esters



INANOIL

Optimisation results – Alkylesters and Waxes

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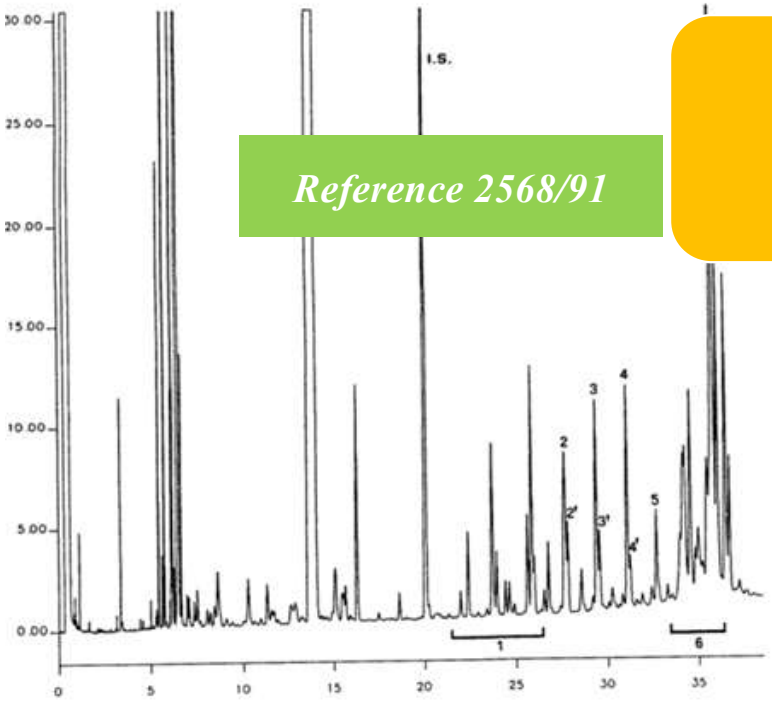


Reference 2568/91

Reference cromatograms comparison

Waxes

I.S. = Lauryl arachidate

1 = Diterpenic esters

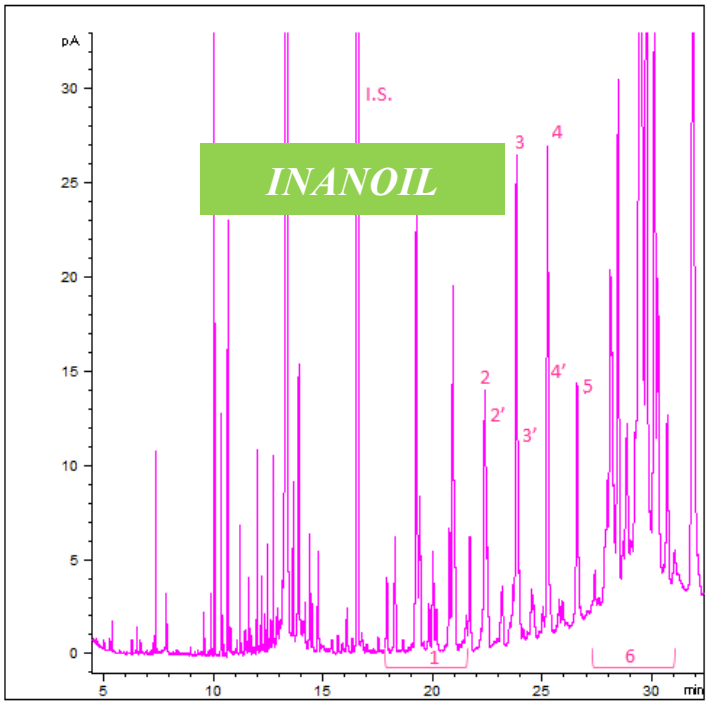
2+2′ = C_{40} esters

3+3′ = C_{42} esters

4+4′ = C_{44} esters

5 = C_{46} esters

6 = Sterol esters and triterpene alcohols



INANOIL

Optimisation results – Sterols and alcohols

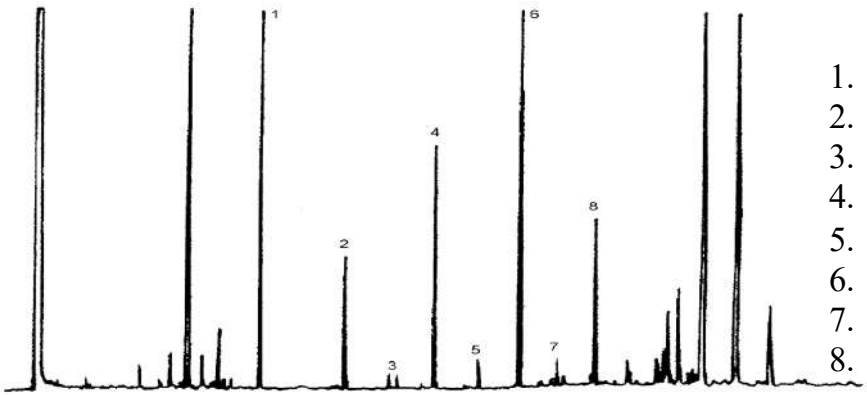
ANNEX V

DETERMINATION OF THE COMPOSITION AND CONTENT OF STEROLS AND TRITERPENES DIALCOHOLS BY CAPILLARY-COLUMN GAS CHROMATOGRAPHY

Reference cromatograms comparison

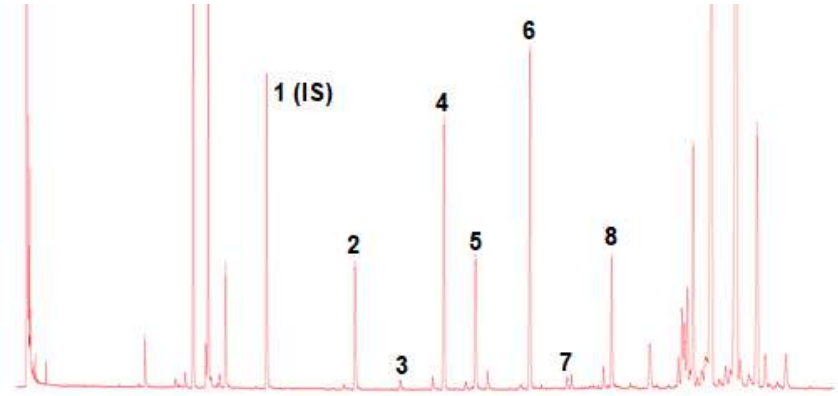
Aliphatic Alcohols

Reference 2568/91



1. Eicosanol (IS)
2. Decosanol
3. Tricosanol
4. Tetracosanol
5. Pentacosanol
6. Hexacosanol
7. Heptacosanol
8. Octacosanolo

INANOIL



Optimisation results – Sterols and alcohols

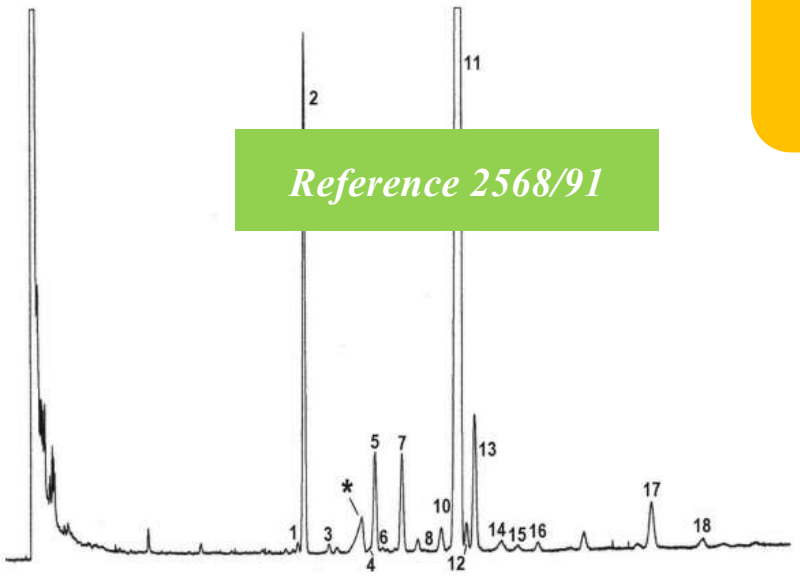
ANNEX V

DETERMINATION OF THE COMPOSITION AND CONTENT OF STEROLS AND TRITERPENES DIALCOHOLS BY CAPILLARY-COLUMN GAS CHROMATOGRAPHY

Reference cromatograms comparison

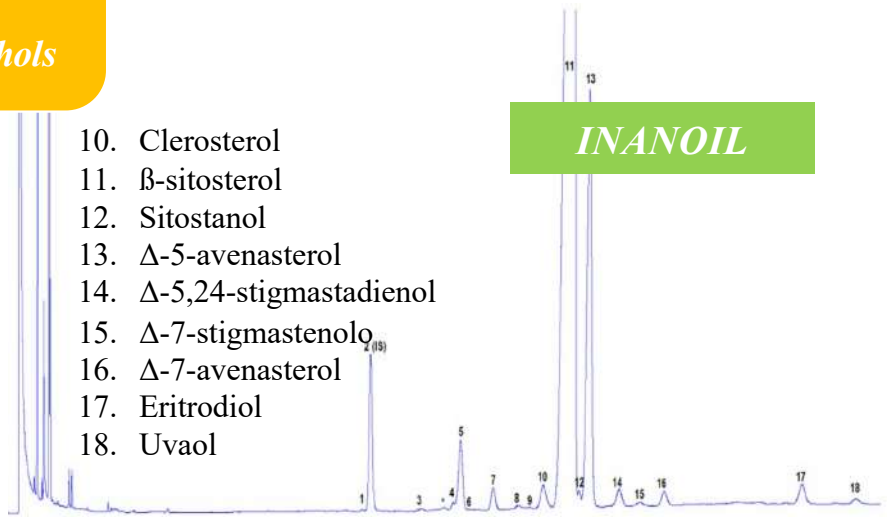
Sterols, triterpenyl dialcohols

Reference 2568/91



1. Cholesterolo
2. Cholestanol (IS)
3. Brassicasterol
* Ergosterolo
4. 24-methylencholesterol
5. Campesterolo
6. Campestanol
7. Stigmasterol
8. Δ-7-campesterol
9. Δ-5,23-stigmastadienol
10. Clerosterol
11. ß-sitosterol
12. Sitostanol
13. Δ-5-avenasterol
14. Δ-5,24-stigmastadienol
15. Δ-7-stigmastenolo
16. Δ-7-avenasterol
17. Eritrodiol
18. Uvaol

INANOIL



Optimisation results – Stigmastadienes

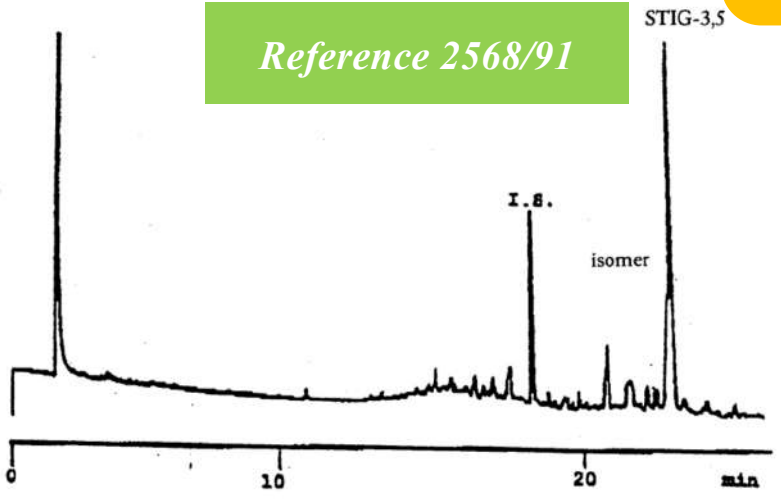
ANNEX XVII

METHOD FOR THE DETERMINATION OF STIGMASTADIENES IN VEGETABLE OILS

Reference cromatograms comparison

Stigmastadienes

Reference 2568/91



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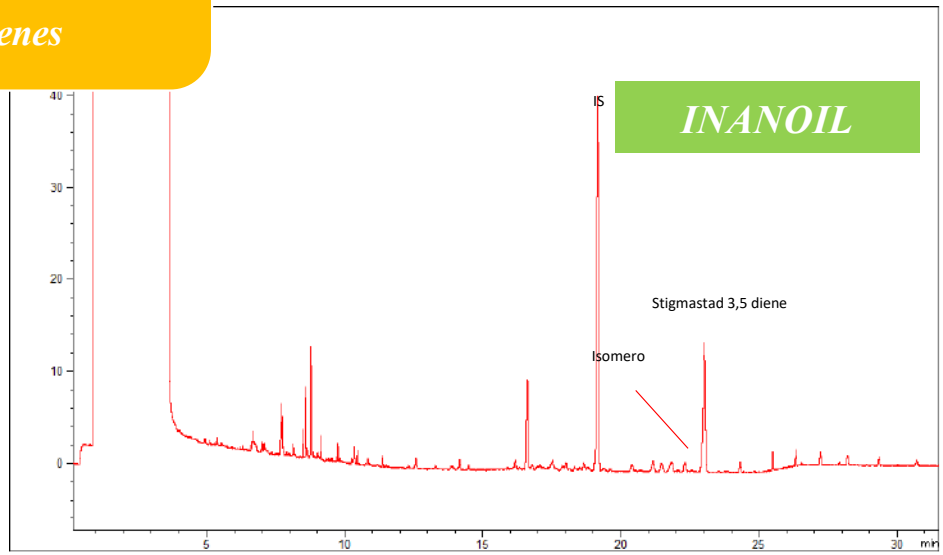

Validation Results

Reference Sample: Ring Test «RT62»
(Camera di commercio di ROMA)
Blend od virgin and refined olive oil

Validation approach:

- **Batches of 10 replicates – Complete automation**
- **Evaluation of Average, CV_r%, BIAS%**

Validation results – Alkylesters and Waxes

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Accuracy and Precision

Validazione CERE

#	mg/kg (sommatoria)
1	225.8
2	216.9
3	215.2
4	215.5
5	227.3
6	215.1
7	224.2
8	224.7
9	215.8
10	224.0

media	dev. St.
220.5	5.1
Valore di riferimento (RT62) Cere = 221.0	
bias% 0.25%	CV_r% 2.32%

Validazione Alchilesteri

#	mg/kg FAEE (sommatoria)
1	36.6
2	35.6
3	36.0
4	35.5
5	35.0
6	35.2
7	35.0
8	35.0
9	35.2
10	35.0

media	dev. St.
35.4	0.53
Valore di riferimento (RT62) FAEE = 36.2	
bias% 2.18%	CV_r% 1.51%

Validation results – Stigmastadienes

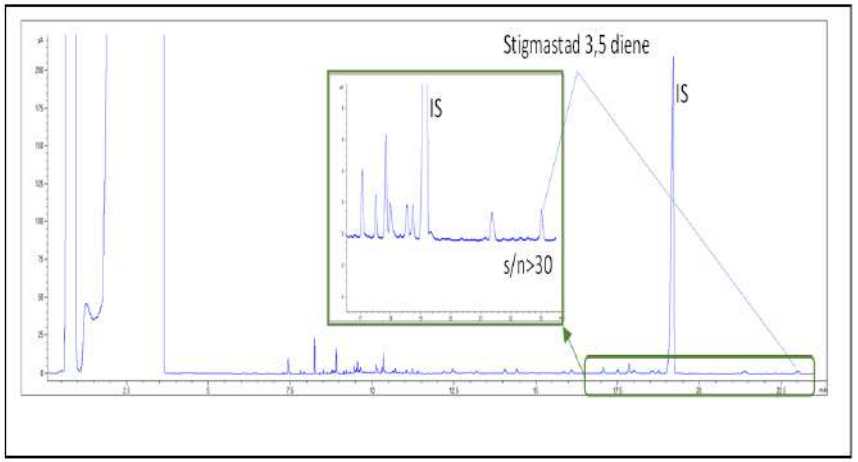
SRA INSTRUMENTS ANALYTICAL SOLUTIONS

Accuracy and Precision

Validazione Stigma RT62

#	mg/kg
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

media	dev. St.
3.70	0.04
Valore di riferimento (RT62) Stigma = 3.70	
bias% 1.66%	CV_r% 1.16%



Validation results – Sterols and alcohols

Accuracy and Precision

Validazione Alcoli Alifatici

#	mg/kg (sommatoria)
1	258.5
2	265.5
3	267.0
4	263.2
5	262.4
6	259.0
7	261.9
8	256.1
9	269.3
10	262.0

media	dev. St.
262.5	4.0
Valore di riferimento (RT62) Alcoli = 258.7	
bias%	CV_r%
1.46%	1.53%

Validazione Steroli e Dialcoli Triterpenici

#	mg/kg steroli totali
1	1464
2	1383
3	1419
4	1398
5	1408
6	1428
7	1409
8	1439
9	1453
10	1420

media	dev. St.
1422	25
Valore di riferimento (RT62) Steroli = 1447	
bias%	CV_r%
1.73%	1.74%

Validation results – Sterols and alcohols

Accuracy and Precision

#	Colesterolo	Brassicasterolo	Campesterolo	Stigmasterolo	Δ-7-stigmastenolo	Eritrodiolo Uvaol	ß-sitosterolo (totale)
1	0.125	0.047	4.22	1.69	0.610	5.76	92.7
2	0.139	0.045	4.21	1.76	0.537	5.40	92.7
3	0.125	0.047	4.18	1.76	0.596	5.75	92.7
4	0.138	0.044	4.15	1.77	0.566	5.42	92.8
5	0.139	0.045	4.14	1.75	0.570	5.44	92.7
6	0.128	0.056	4.20	1.75	0.585	5.86	92.7
7	0.121	0.053	4.17	1.76	0.579	5.52	92.7
8	0.131	0.057	4.24	1.86	0.553	5.96	92.6
9	0.118	0.044	3.91	1.76	0.597	5.42	93.0
10	0.125	0.047	4.20	1.71	0.573	5.75	92.7
RT62	0.136	0.045	3.95	1.81	0.552	5.368	92.5
Avg	0.129	0.048	4.16	1.76	0.577	5.628	92.7
CVr%	5.98%	10.27%	2.25%	2.47%	3.76%	3.72%	0.11%
BIAS%	5.10%	7.67%	5.31%	3.02%	4.45%	4.83%	0.28%

INANOIL impruvements – Materials/additional devices

	Alkylesters and Waxes	Stigmastadienes	Sterols and Alcohols
Materials			
Generic lab glassware	NOT Needed	NOT Needed	NOT Needed
Flasks	NOT Needed	NOT Needed	NOT Needed
LC glass columns	NOT Needed	NOT Needed	NOT Needed
Test tubes	NOT Needed	NOT Needed	NOT Needed
Separating funnels	NOT Needed	NOT Needed	NOT Needed
TLC sheets			NOT Needed
TLC chambers			NOT Needed
UV Lamp			NOT Needed
Desiccator			NOT Needed
Additional devices			
Rotavapor (or equivalent)	NOT Needed	NOT Needed	NOT Needed
Nitrogen blowing device	NOT Needed	NOT Needed	NOT Needed
Muffle	NOT Needed	NOT Needed	NOT Needed

INANOIL impruvements – Chemicals

	Alkylesters and Waxes		Stigmastadienes		Sterols and Alcohols	
Chemicals (estimated average amounts for a 10 sample batch)						
Chemical	EU Method → **INANOIL**	% Saving - **amount saved**	EU Method → **INANOIL**	% Saving - **amount saved**	EU Method → **INANOIL**	% Saving - **amount saved**
Activate silica	150 g → **not needed**	100% - **150 g**	150 g → **not needed**	100% - **150 g**	-	-
Organic solvents	3000 ml → **250 ml**	92% - **2750 ml**	3500 ml → **200 ml**	95% - **3200 ml**	3000 ml → **200 ml**	93% - **2800 ml**
Hydro(alcoholic) reagents for saponification	-	-	4000 ml → not needed	100% - **4000 ml**	same as EU Method **(so far)**	-

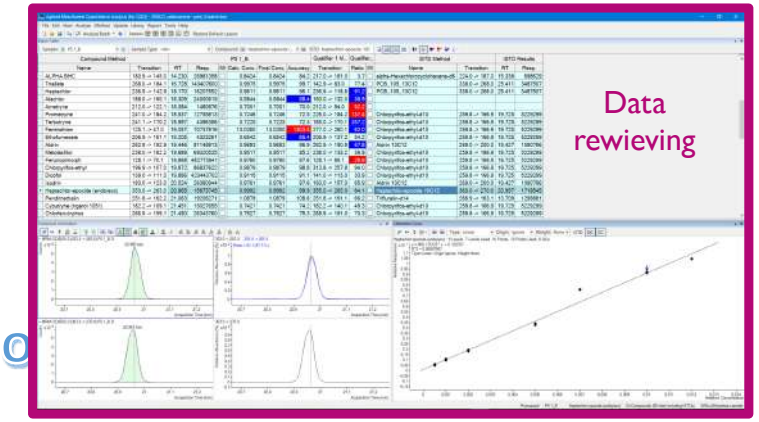
INANOIL impruvements – Lab technician commitment

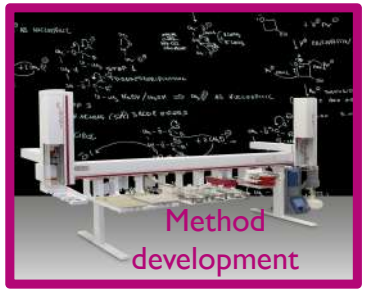
	Alkylesters and Waxes		Stigmastadienes		Sterols and Alcohols	
	Operator working time (estimated average for a 10 sample batch) - hours					
	EU Method → **INANOIL**	% Saving - **amount saved**	EU Method → INANOIL	% Saving - **amount saved**	EU Method → **INANOIL**	% Saving - **amount saved**
Overall (including GC analysis)	12 → **8**	33% - **4**	15→ 9.5	36% - **5.5**	10 → **4**	60% - **6**
Sample prep only	4 → **0.5**	87% - **3.5**	10 → 0.5	95% - **9.5**	7 → **0.5**	92% - **6.5**

INANOIL impruvements – Final Remarks

Aimed to….

- Drastically reduce the consumption of solvents
- Limit the intervention of expert operators to a few minutes, reducing the entire sample-prep to the simple dilution of the initial sample (eventually saponified)
- Eliminate the use of glassware and consumables
- Effectively cancel the incidence of random errors

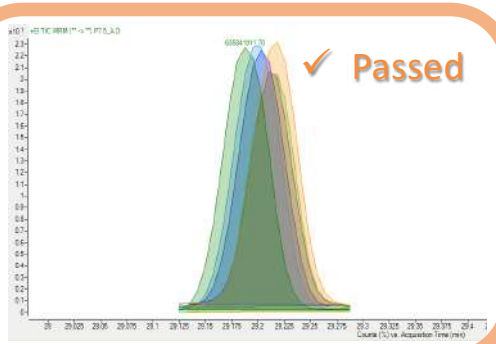




LESS
solvent exposure

… take care of analyst's health, and let him to exploit his skills «away from the hood»

Totally automated sample-prep and analysis



SRA INSTRUMENTS
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Analizzatori INANOIL

PARTE 1:

Me… …ti… …teri e Cere

PARTE 2:

Ster… …A… …oli

PARTE 3:

Stigmastadieni

7 luglio

Aknowledgments

SRA INSTRUMENTS
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Centro Analisi Biochimiche sas

srainstruments.com

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Piattaforma INANOIL per la determinazione di Steroli e Alcoli

PARTE 2

SEPARAZIONE DELLE FRAZIONI DEI COMPOSTI ALCOLICI

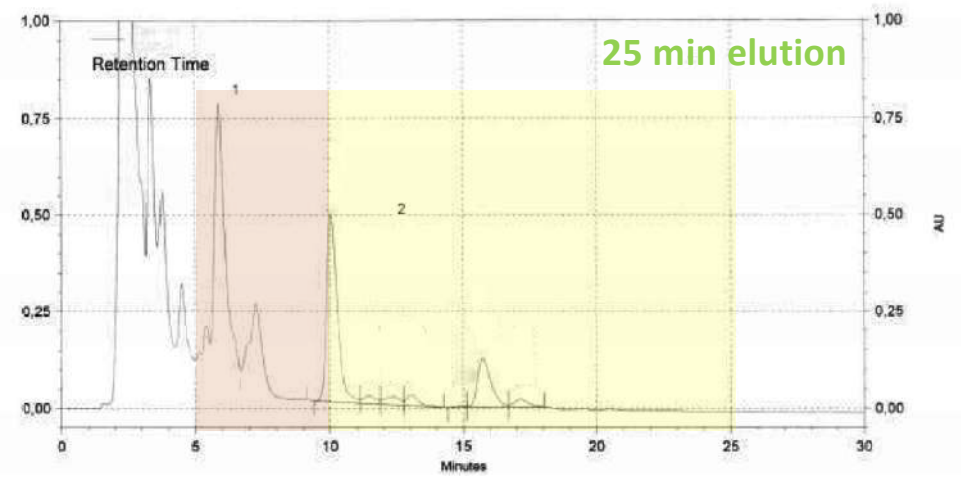
1. OGGETTO

L'insaponificabile preparato come descritto nella parte 1 è frazionato nei diversi composti alcolici, alcoli alifatici, steroli e dialcoli triterpenici (eritrodiolo e uvaolo).

2. PRINCIPIO

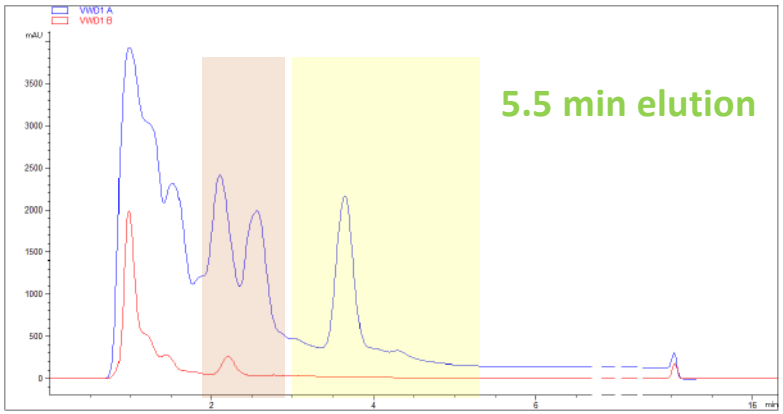
L'insaponificabile può essere frazionato per mezzo della cromatografia basica su strato sottile (metodo di riferimento), rivelato per poi raschiare ed estrarre le corrispondenti bande. Come metodo alternativo, la separazione può essere realizzata mediante HPLC con colonna di gel di silice e rivelatore UV raccogliendo le diverse frazioni. Gli alcoli alifatici e triterpenici nonché gli steroli e i dialcoli triterpenici sono isolati insieme.

HPLC separation



ALCOHOLIC FRACTION

STEROLIC FRACTION



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Piattaforma INANOIL per la determinazione di Steroli e Alcoli

ALCOHOLIC FRACTION

STEROLIC FRACTION

ALCOHOLIC FRACTION

STEROLIC FRACTION