



TRICAP 2009

ThRee-way methods In Chemistry And Psychology

Two- and three-way methods for the resolution of overlapping peaks in the determination of acid phenols in olive oil by HPLC-DAD

Federico Marini, Antonio D'Aloise, Antonio Magrì,
Andrea Magrì, Remo Bucci

DIPARTIMENTO DI CHIMICA



SAPIENZA
UNIVERSITÀ DI ROMA



Overview

- Motivation
- Experimental setup
- Chemometric analysis
- Preliminary conclusions and future work

Polyphenols in Extra virgin olive oil: *properties*

- During the last years, the benefits of mediterranean diet for the prevention of cardiovascular diseases and cancer have been thoroughly investigated.
- Many studies have demonstrated the effectiveness of natural antioxidants → *polyphenols*.
- These species, acting as **radical scavengers** are powerful inhibitors of the formation of atherosclerotic plaques and oxygen reactive species (cause of the “fat-related” neoplastic diseases, often correlated to a too high assumption of ω -6 polyunsaturated acids).
- Extra virgin olive oil, due to its composition (70% oleic acid and significant amount of phenolic compounds) is considered to be a nutritionally important food.

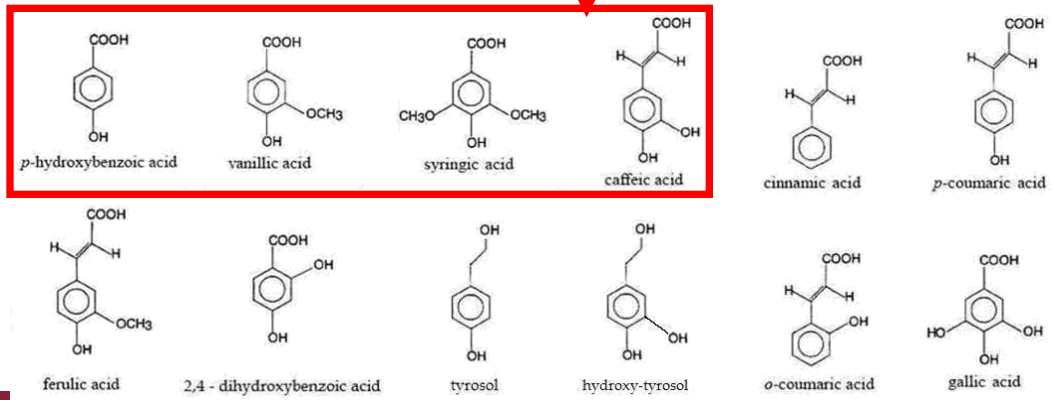
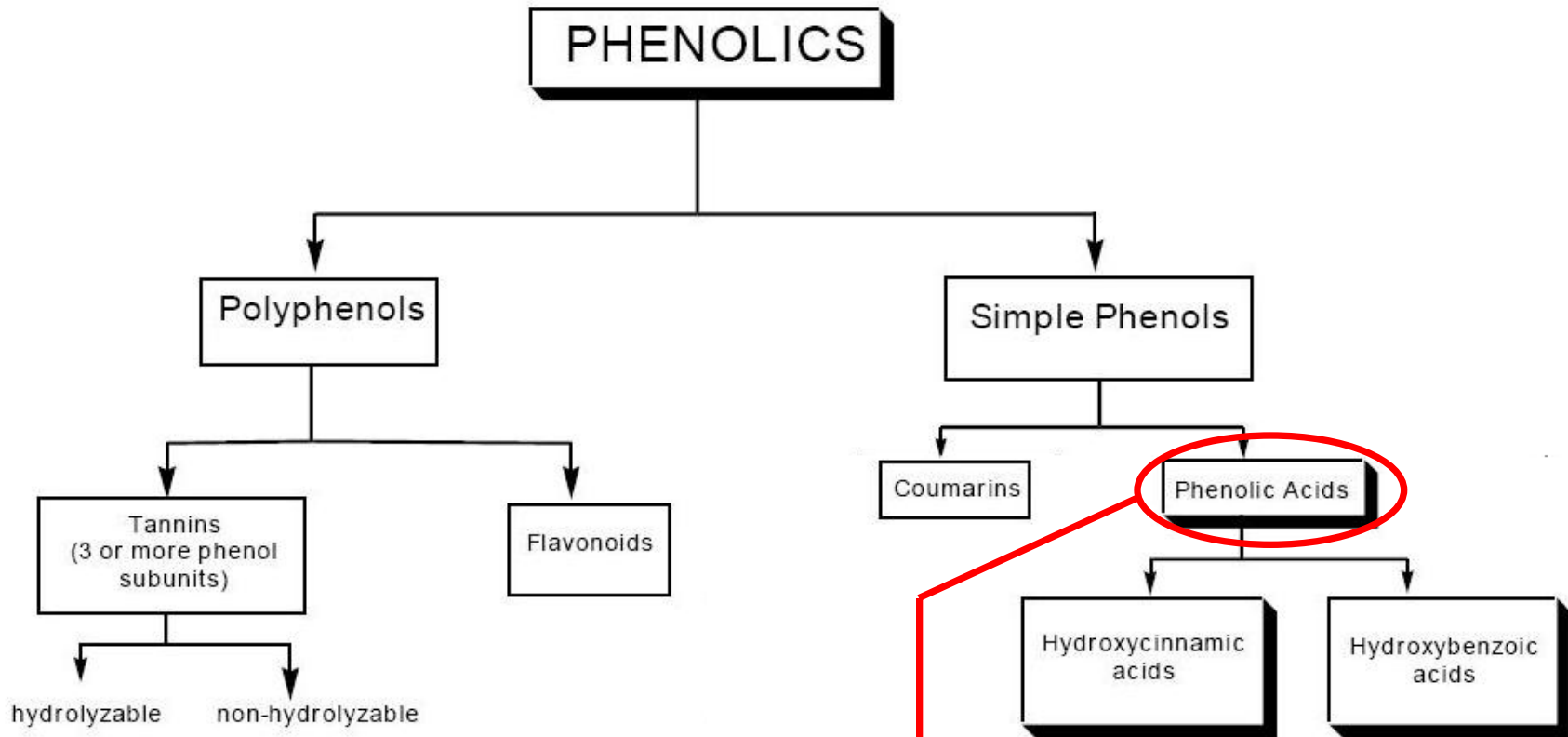
Extra virgin olive oil composition

GLYCERIDES:

- Triglycerides (97-98%)
- Diglycerides (2-3%)
- Monoglycerides (0.1-0.2%)

MINOR COMPONENTS:

- Hydrocarbons (150-180 mg/100g)
- Aliphatic alcohols (10-20 mg/100g)
- Triterpenic alcohols (100-300mg/100g)
- Sterols (80-260 mg/100g)
- Phenols (20-900 mg/Kg)
- Tocopherols (50-300 mg/Kg)



Analysis of phenolic compounds

- Aspecific colorimetric analysis by the Folin-Ciocalteu (total phenols)

or

- *Liquid – liquid* or solid phase extraction by SPE cartridge (C₁₈, diol, aminic, phenylic)
+
RP-HPLC gradient analysis coupled to UV, fluorescence, DAD or MS detection (rarely GC or CE).

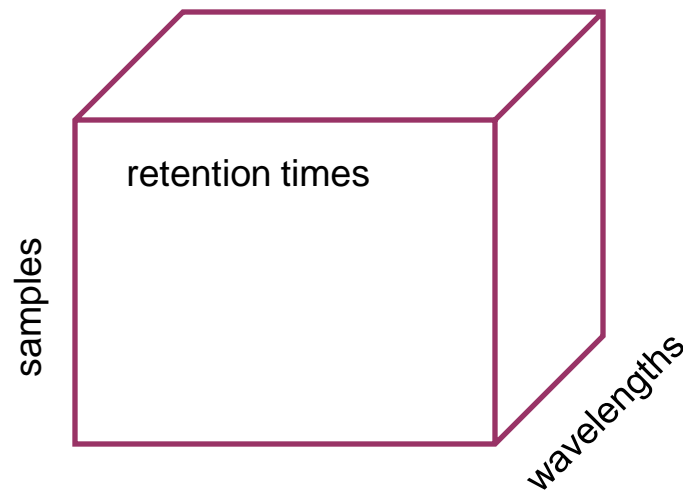
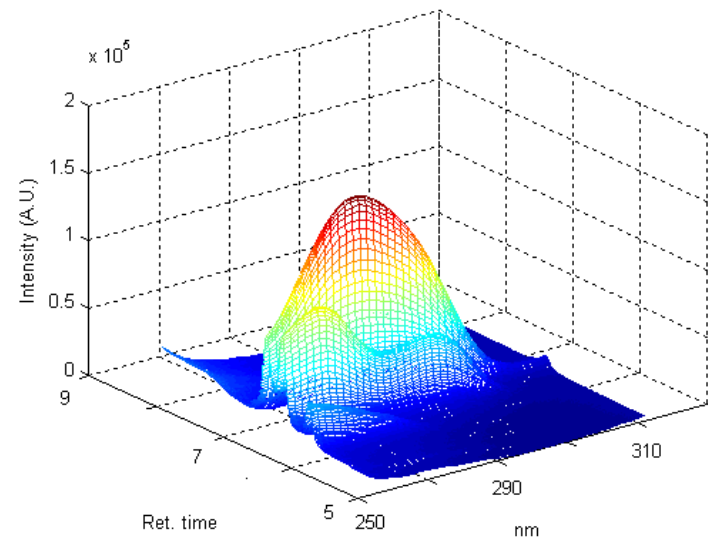
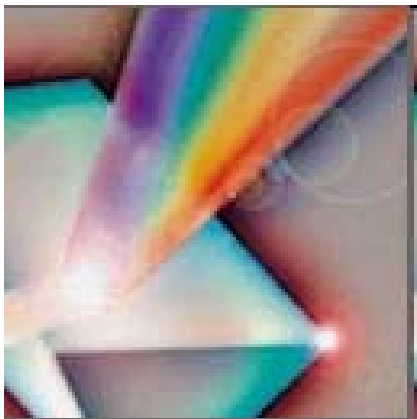
Disadvantages: Long analytical times even with gradient
(60-70 mins for the analysis of all phenolic acids)

Our goal

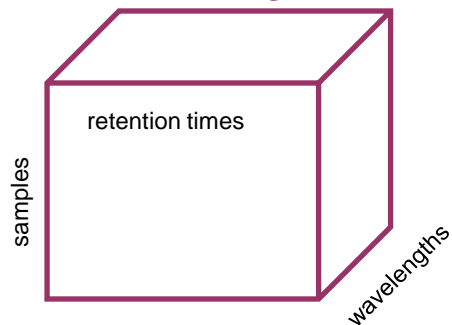
Use of second order techniques for the a posteriori resolution of overlapping chromatographic peaks, allowing to achieve:

- Easier sample preparation
- Shorter analytical times
- (possibility of using shorter columns → Fast LC)
- Lower consumption of solvents.

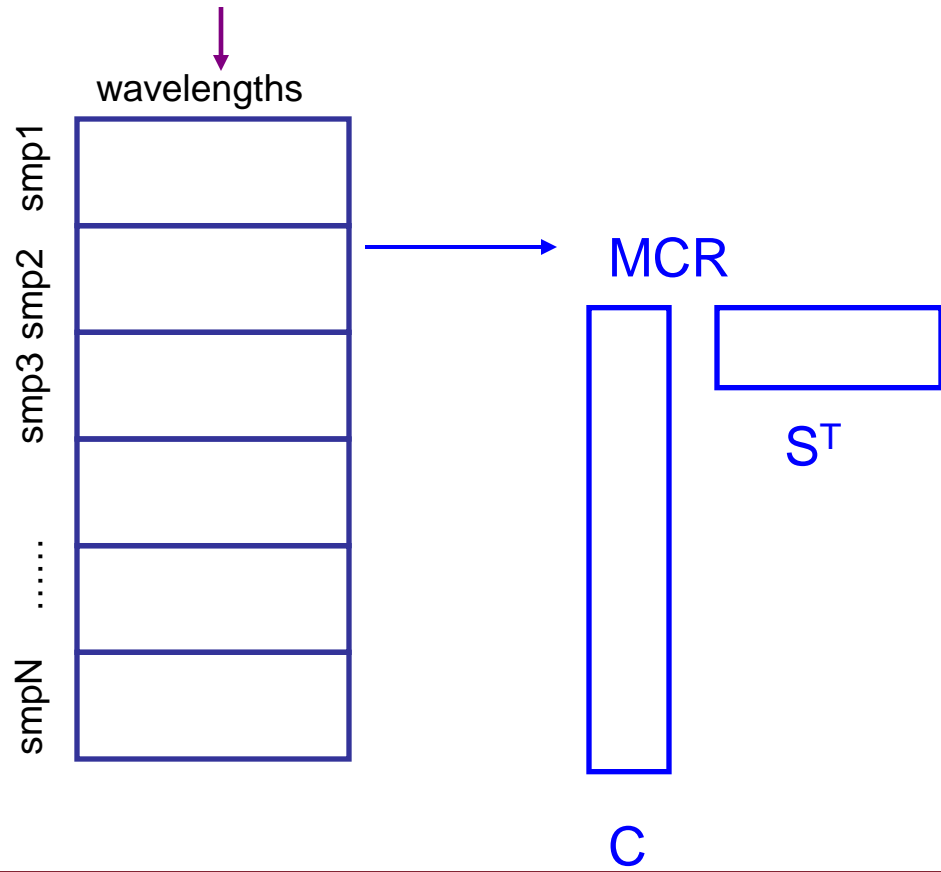
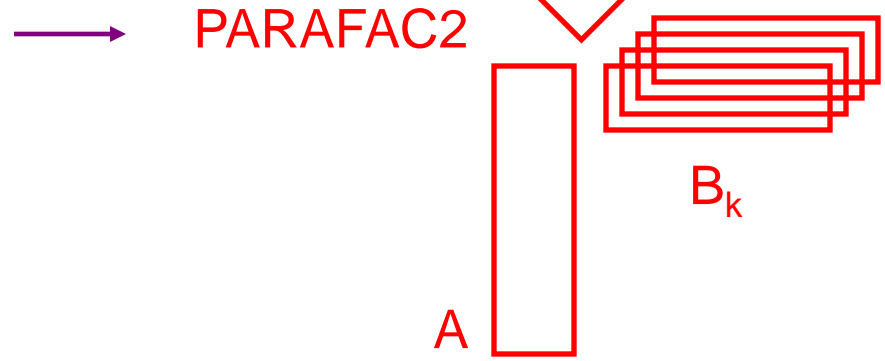
HPLC-DAD



Data analysis



- Shift in the retention times
- Changes in peak shapes



EXPERIMENTAL

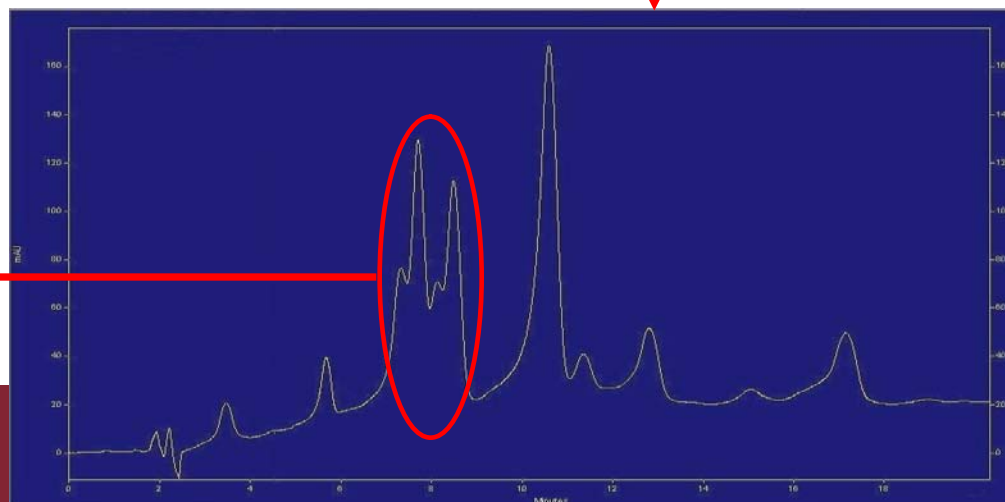
RP-HPLC ANALYSIS (C_{18} column i.d. 0,46 × 15 cm)

ELUTION GRADIENT: mobile phase H_2O/CH_3OH acidified with 1% CH_3COOH ; → 0–2 min. 95 to 75 % H_2O , 2-10 min. from 75 to 60%, 10-19 min. to 60 al 40%.

N° esperimento	idrossi-tirosolo	tirosolo	ac. idrossi-benzoico	ac. vanillico	ac. siringico	ac. caffeico	ac. ferulico	vanillina	ac. m-cumarico	ac. o-cumarico	ac. cinnamico
1	+	+	+	+	+	+	+	+	+	+	+
2	-	-	+	+	+	-	-	-	-	+	+
3	+	+	-	+	+	-	-	+	-	-	-
4	-	+	-	+	-	+	-	-	+	-	+
5	-	+	-	-	+	+	+	-	-	+	-
6	+	-	+	+	-	+	+	-	-	-	-
7	-	+	+	-	-	-	+	+	-	-	+
8	-	-	+	-	+	+	-	+	+	-	-
9	-	-	-	+	-	-	+	+	+	+	-
10	+	+	+	-	-	-	-	-	+	+	-
11	+	-	-	-	-	+	-	+	-	+	+
12	+	-	-	-	+	-	+	-	+	-	+

Screening 11 factors
PLACKETT-BURMAN design

4 overlapping peaks



CHROMATOGRAPHIC METHOD

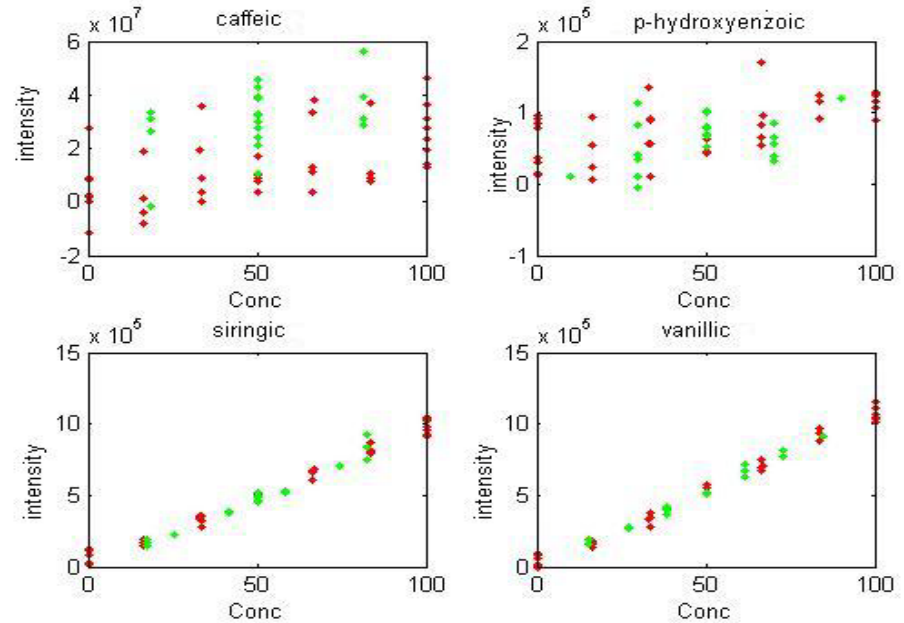
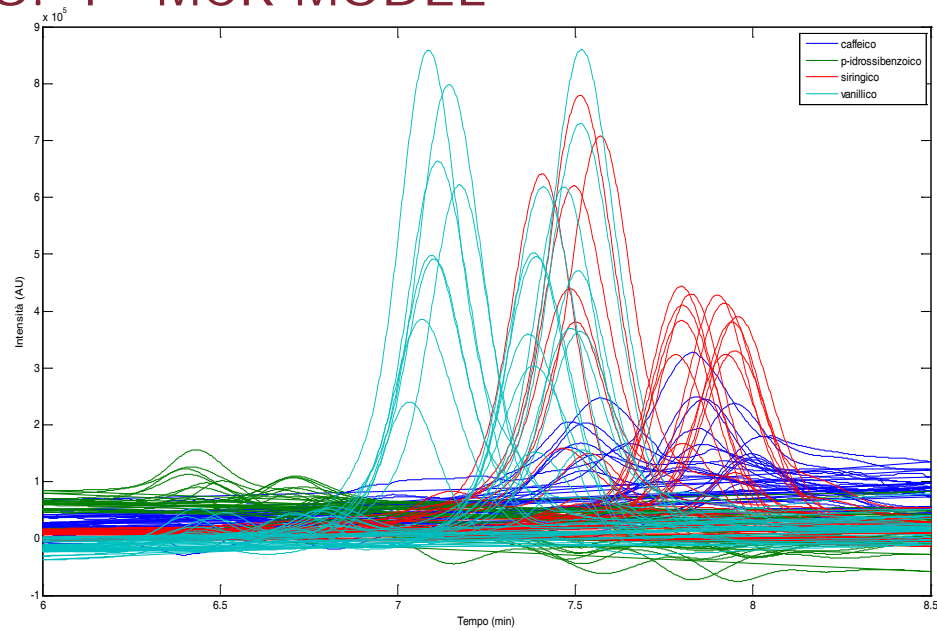
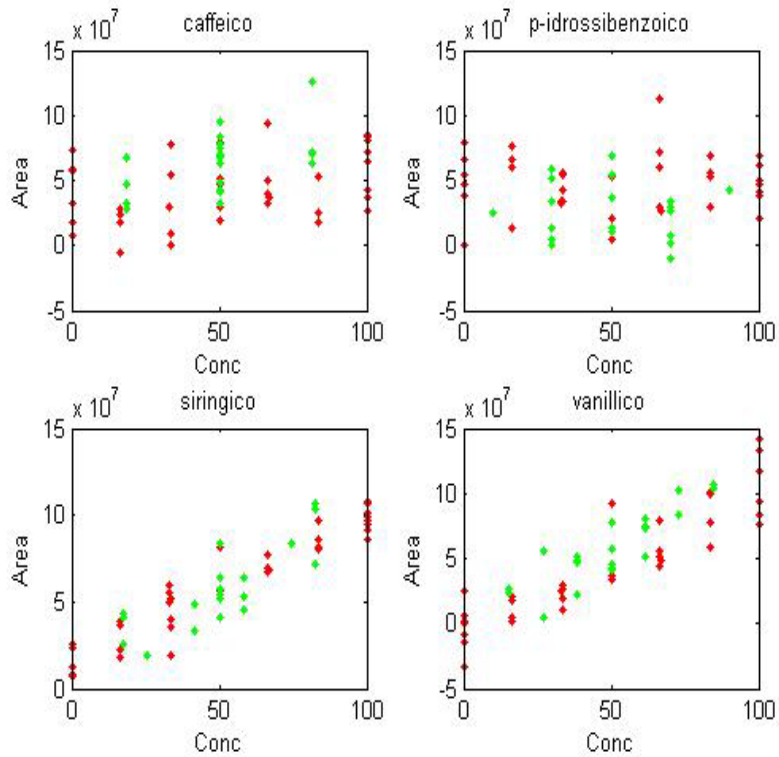
Several kinds of extractive phases were tested (Octadecyllic SPE, cyano, silicic, polymeric and phenilic; LLE using different methanol to water ratios). Eventually, LLE was chosen:

- 3 g oil
- + 1.5 ml exane;
- extraction with 2 ml di metanolo ×2;
- centrifugation for 6 minutes a 3600 rpm;
- evaporation of methanolic phase to dryness;
- dissoution of the dry extract in 200 µL methanol;
- HPLC analysis.

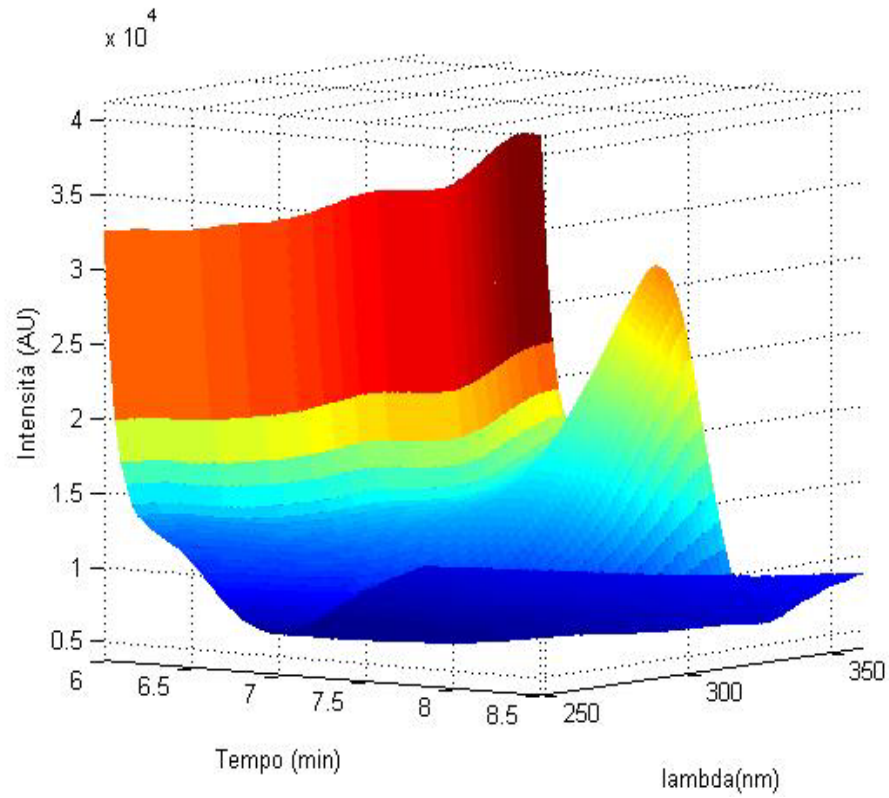
DATA SETS

- 38 standard mixtures were used as ***training set*** and 21 as ***test set***.
- The mixtures were prepared dissolving the opportune amount of standard in a vegetable oil polyphenol free (the best choice was peanut oil) to reproduce the effects of the oil matrix.
- Composition of the training and test matrices was chosen based on two separate onion designs.
- A wavelength range from 248 to 360 nm and a retention time window from 6.5 to 8.5 minutes (corresponding to the four coeluted peaks) were selected, so that the resulting arrays were **$1001 \times 57 \times 38$** for the ***training set*** and **$1001 \times 57 \times 21$** for the ***test set***.

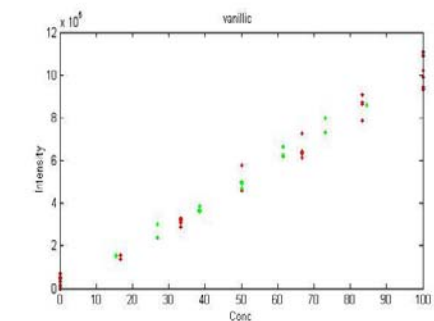
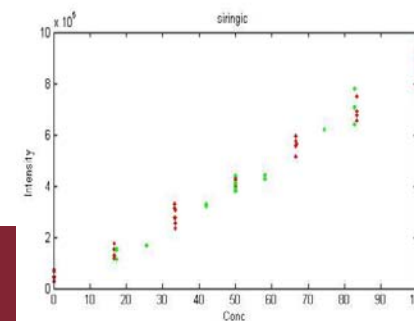
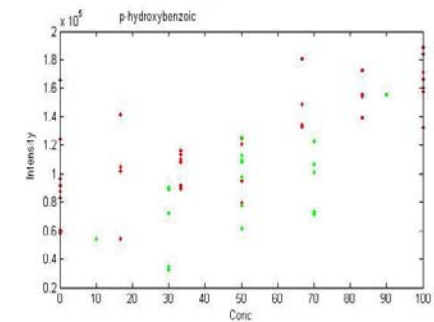
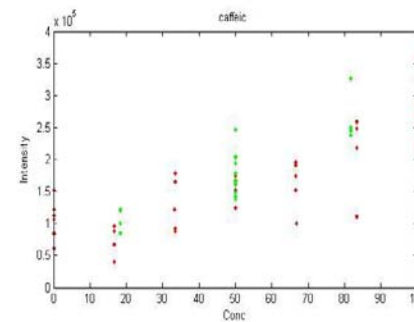
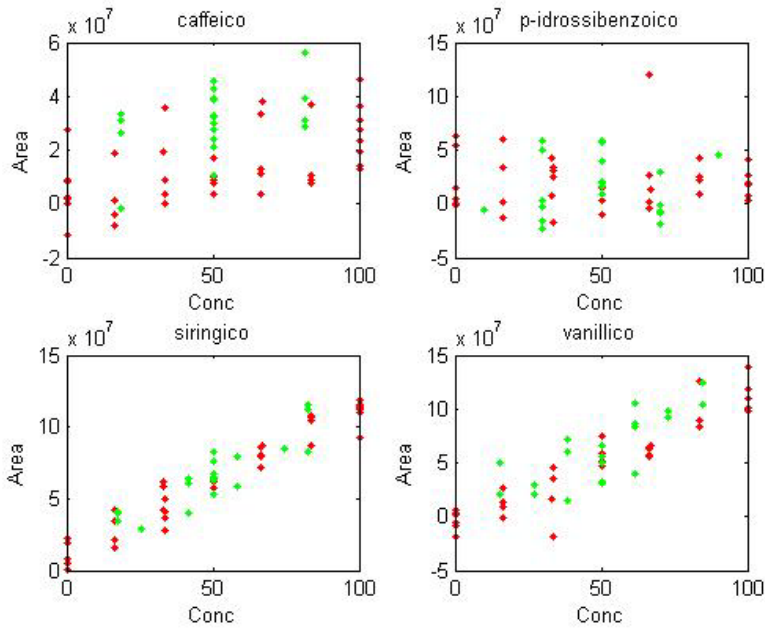
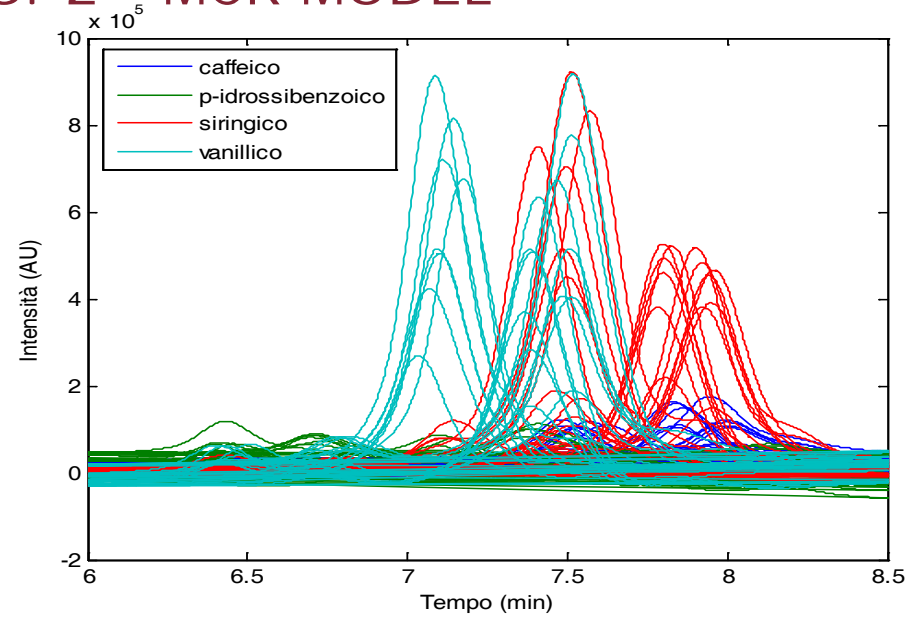
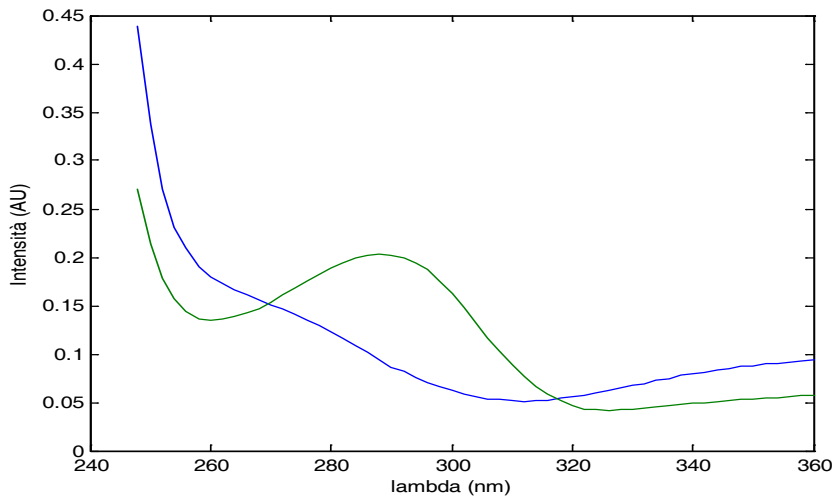
RESOLUTION OF THE 4 ANALYTES: 1st MCR MODEL



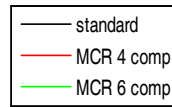
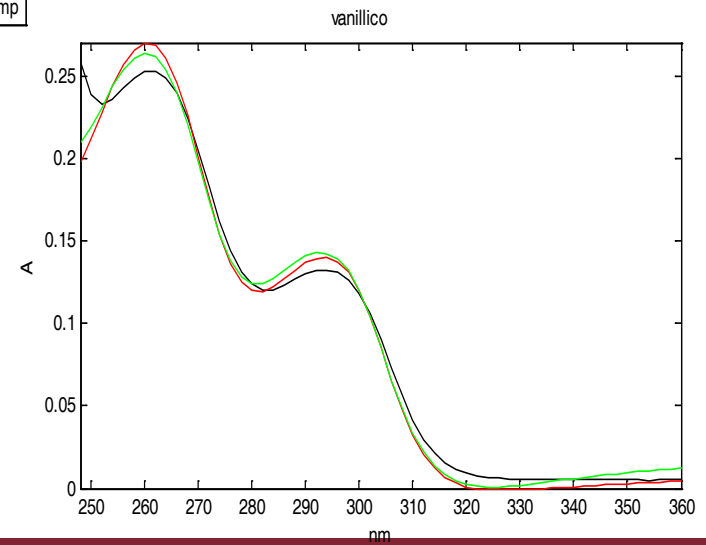
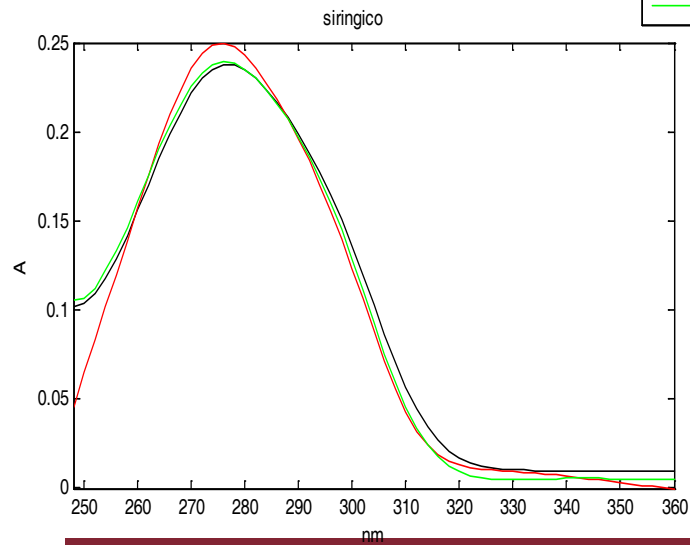
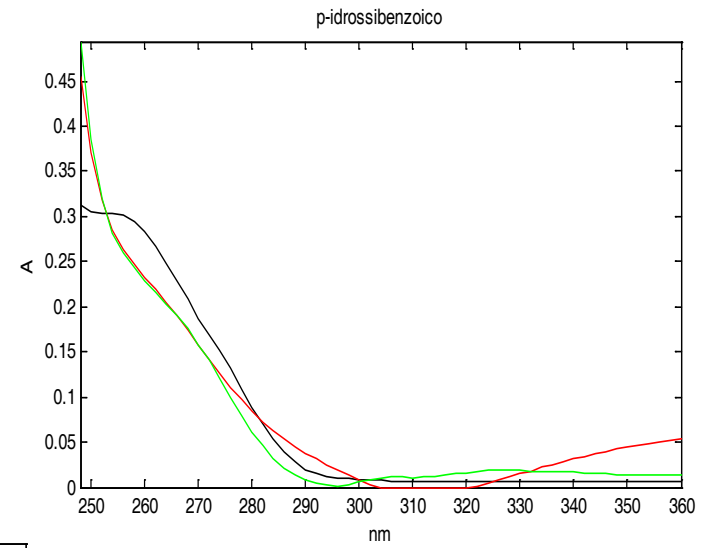
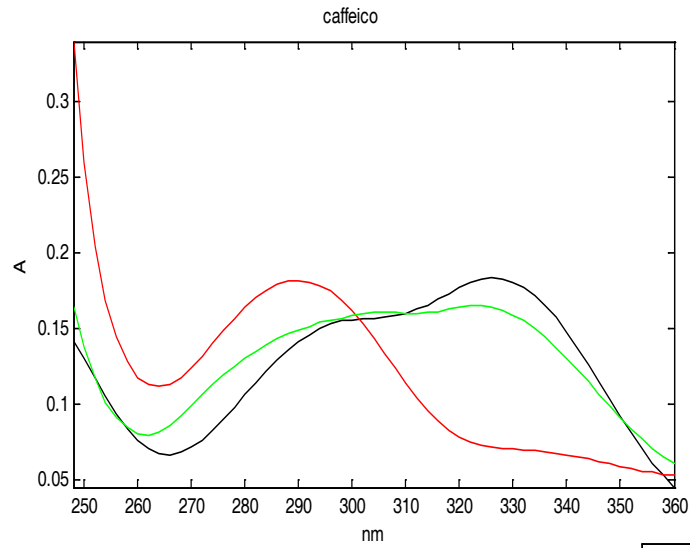
Baseline and oil interferents effect



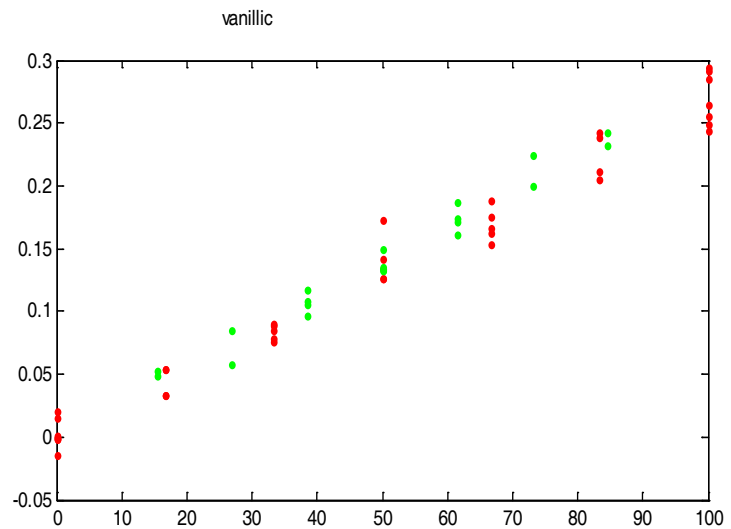
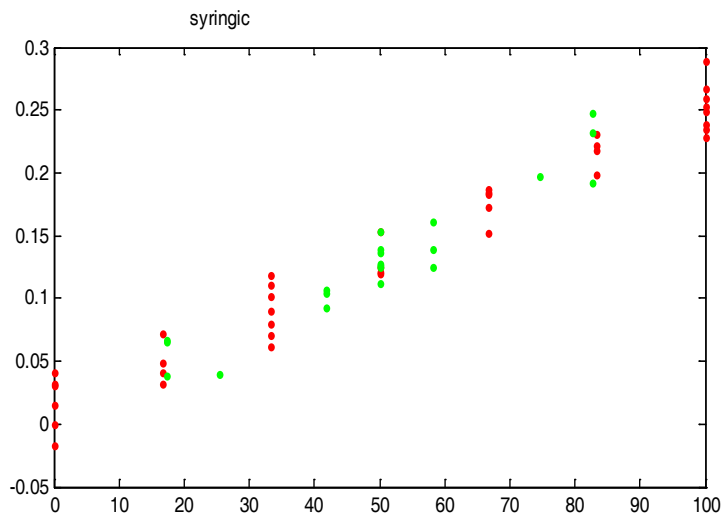
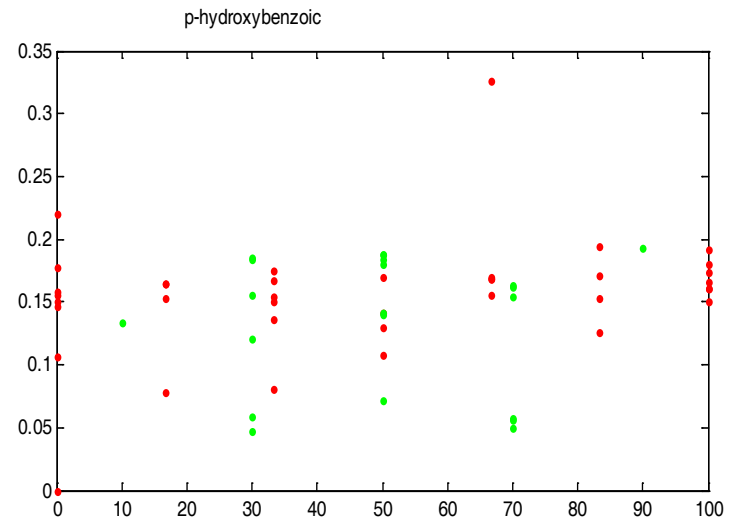
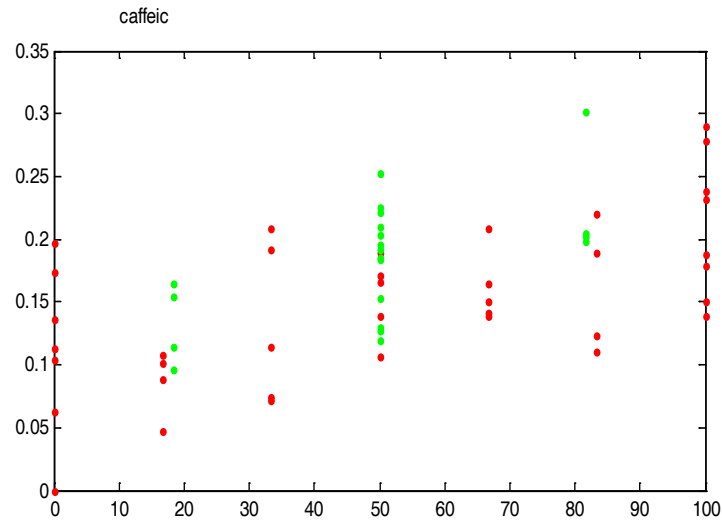
RESOLUTION OF THE 4 ANALYTES: 2nd MCR MODEL



MCR: spectra



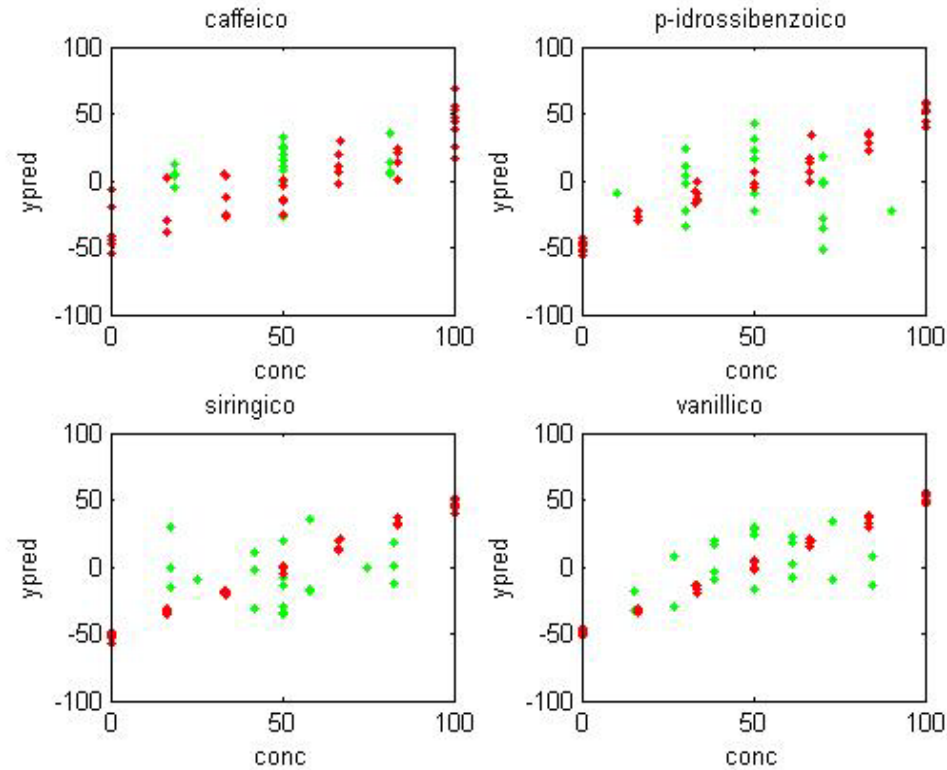
RESOLUTION OF 4 PEAKS: PARAFAC2 MODEL



R² FOR THE MODELS

	MCR 6 c.		PARAFAC2	
	Training	Test	Training	Test
caffeic	0.5633	0.3031	0.6648	0.5629
p-hydroxybenzoic	0.0069	0.0012	0.1871	0.0506
syringic	0.9868	0.9668	0.9412	0.9277
vanillic	0.9644	0.9441	0.9561	0.9318

CALIBRATION OF 4 ANALYTES: 4 N-PLS MODEL



- **7 LV caffeic**
- **9 p-hydroxybenzoic**
- **5 syringic**
- **6 vanillic**

CONCLUSIONS

- Both PARAFAC2 and MCR give comparable results on this dataset, both in modeling and on independent test set.
- Syringic and vanillic acids are best modeled, caffeic is not modeled well, p-hydroxic benzoic can't be modeled at all
- N-PLS preliminary results overfitted.
- Better analysis of matrix effects and S/N ratio should be carried out.

Thanks for your attention!

