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APPLICATION OF ADVANCED SEPARATION TECHNIQUES IN WINE QUALITY CONTROL

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INTRODUCTION

HOW MANY GRAPE VARIETIES ARE GROWN?

Several thousand to be precise, but a **few hundred** are actually used for wine making.

The vine plant can produce fruit for up to 100 years.

…y starting with the highest

…ariety of soil types,

…, Asia, Mediterranean and
…land, most of North America

GRAPE VARIETIES IN R. MACEDONIA

- **Red Grape Varieties: Vranec, Stanušina, Kratošija,** Merlot, Pinot Noir, Cabernet Sauvignon, Cabernet Franc, Karadrka

Stanušina

Vranec

Merlot

- **White Grape Varieties: Smederevka**, **Žilavka**, **Župjanka**, Traminec, Temjanika (Riesling) Chardonnay, Semilion, Sauvignon Blanc, Muscat Ottonel, Grenache Blanc-Belan

Smederevka

Žilavka

Chardonnay

In order to improve the quality of wines, research in viticulture and enology is necessary:

- to improve the grape quality and ripening,
- to select yeast inoculums and enzymes,
- to control the conditions during the malolactic fermentation as well as the aging process.

A sound knowledge of wine chemistry is also necessary.

Chemical Analysis

Several analyses are essential:

- **pH**
- **free and total sulfur dioxide**
- **titratable acidity**
- **reducing sugar**
- **alcohol**
- **protein stability (for whites and low-tannin reds)**
- **potassium bitartrate stability**
- **MLF status, biological stablity**
- **Proper and controlled sensory analysis**

These are the very minimum analyses, <u>ADVANCED ANALYSES NECESSARY!!</u>

ADVANCED ANALYSIS

Determination of:
-specific parameters,
- individual compounds,
- compounds in low concentration….

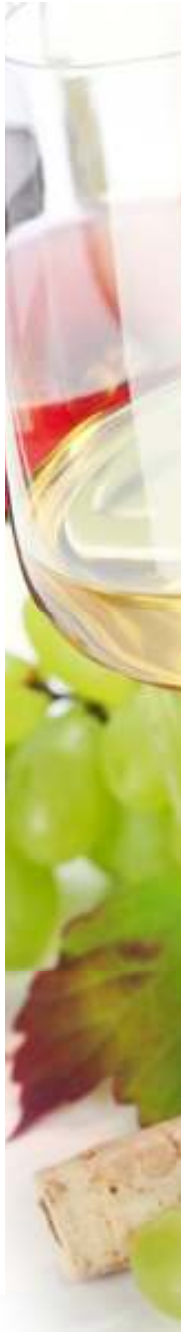
ADVANCED ANALYTICAL TECHIQUES:

-GC- MS (volatile compounds determination: esters, alcohols, terpenes)
- HPLC, CE with DAD or MS (non-volaticle compounds determination: polyphenols, organic acids, carbohydrates, biogenic amines, pesticides…
- MALDI-TOF-MS – identification and structural characterization of big and unknown molecules
- NMR

AROMA COMPOUNDS

- Wine aroma represents a good balance of a several hundred volatile compounds.
- Some volatile compounds originate from the grapes where they are synthesized.
- Most of them are formed during the process of grape must fermentation and afterwards, during the storage of wines.
- All changes of aroma compounds affect the complexity of the aroma profile of wines.

GAS CHROMATOGRAPHY



- Aroma compounds are usually analyzed **by gas chromatography/mass spectrometry (GC/MS)**, as a highly efficient separation technique for volatiles' analysis and for characterization of the wine bouquet.

- GC-MS is also suitable for quantification purposes, using polar column for separation of the components, since it is more sensitive for analysis of components present in a low concentration, as well as, in a complex matrices, as wine is.

- Extraction methods: solid-phase extraction (SPE), solid-phase microextraction (SPME), stir bar sorptive extraction (SBSA), or **Liquid-liquid extraction** methods using organic solvents (dichloromethane), showing high repeatability

Ivanova et al. *Food Analytical Methods*, 5, 1427-1434, 2012
Ivanova et al. *Food and Bioprocess Technology,* 6(6) 1609-1617, 2013

HS-SPME-GC-MS analysis of aroma compounds

An automated HS-SPME combined GC-MS is highly **efficient separation technique** for extraction and separation of wine aroma compounds

✓SPME fiber was used: **DVB/Carboxen/PDMS 50/30, 2** cm stable flex
✓ the samples were equilibrated in the oven of the autosampler at 40°C for 5 minutes
✓ SPME fiber was exposed into the headspace of the sample for 20 minutes at 40°C
✓ transferred to the GC-injector for thermo-desorption at 270°C

Complex aroma profile of Vranec samples

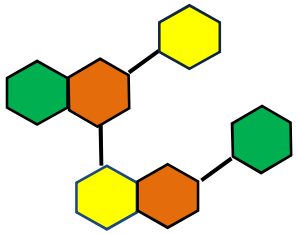
- 18 alcohols: **isoamyl alcohol – dominant alcohold,** followed by 2-methyl-1-butanol, phenylethyl alcohol, isobutyl alcohol and 1-hexanol

- 29 esters: **ethyl acetate** - dominant ester, followed by butanedioic acid diethyl ester, 1-butanol-3-methyl acetate and propanoic acid ethyl ester

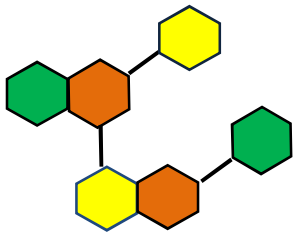
- 7 fatty acids: **octanoic acid** - dominant one, followed by hexanoic acid

- Carbonyl compounds: ***n*-heptanal** and **decanal** were the main compounds

PHENOLIC COMPONENTS



- ✓ Determine the colour, mouth feel, astringency and bitterness of wine.
- ✓ Influence the sensorial characteristics of grape and wine
- ✓ Antioxidant, antimicrobal, anticancerogenic effects, prevention of cardiovascular diseases.

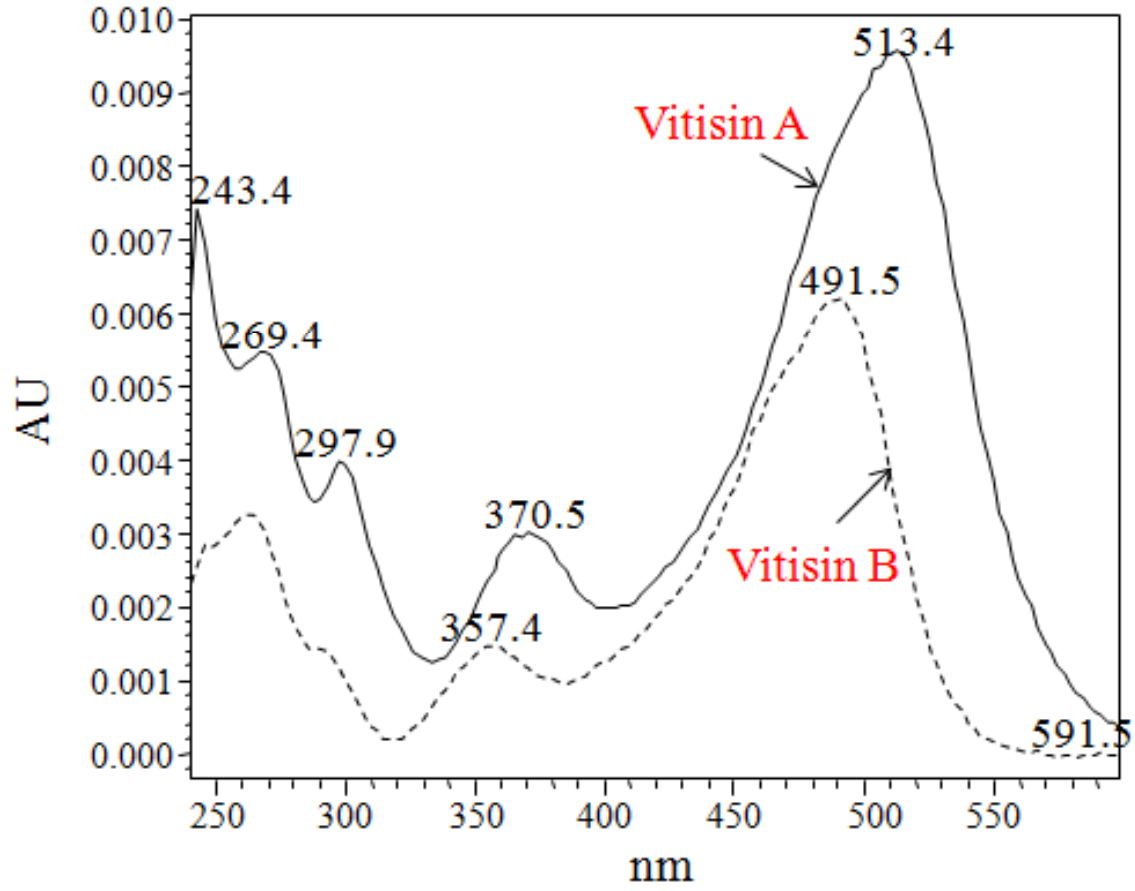


Two groups of polyphenols:

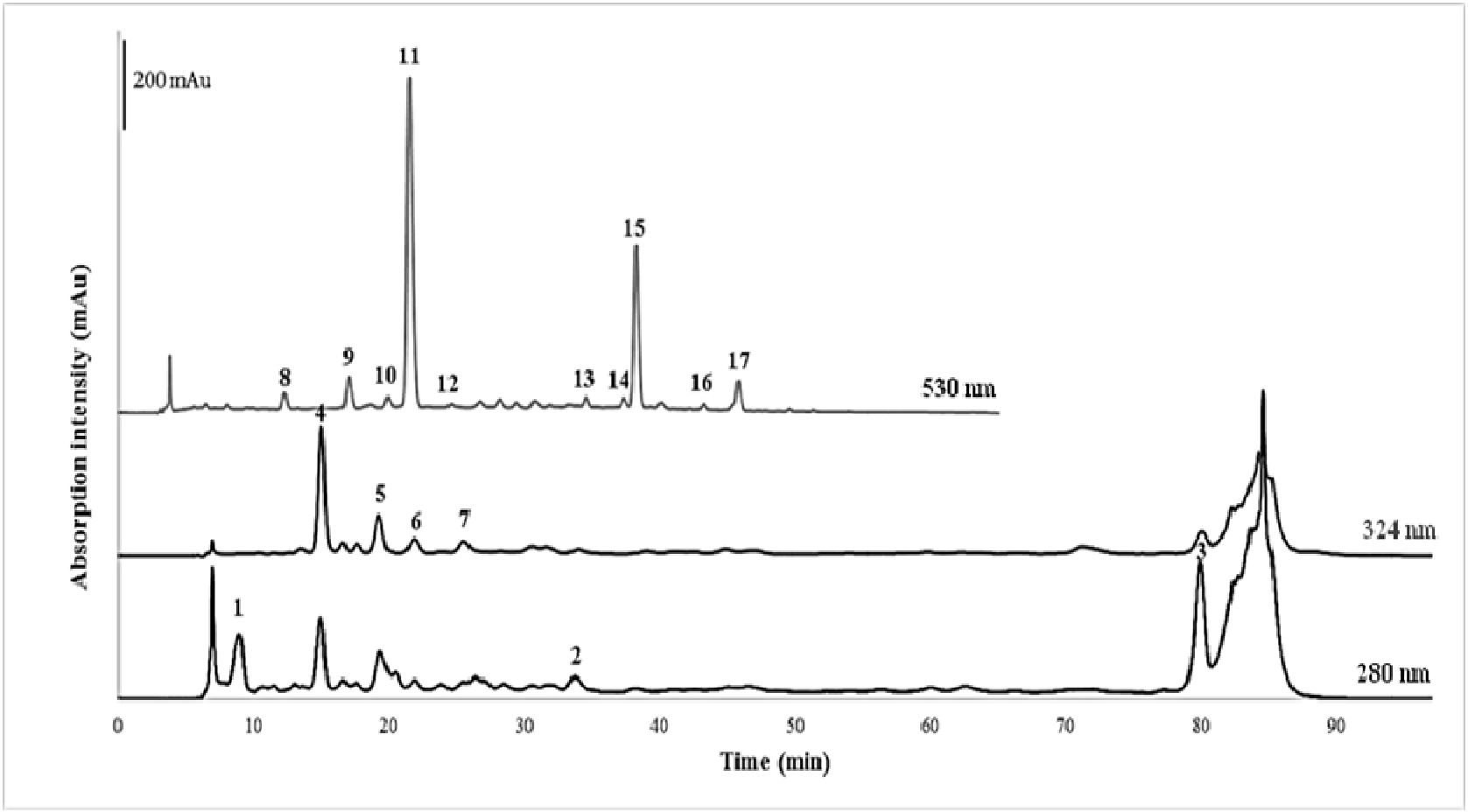
Flavonoids

Non-flavonoids

UV-Vis spectra of vitisin A and vitisin B

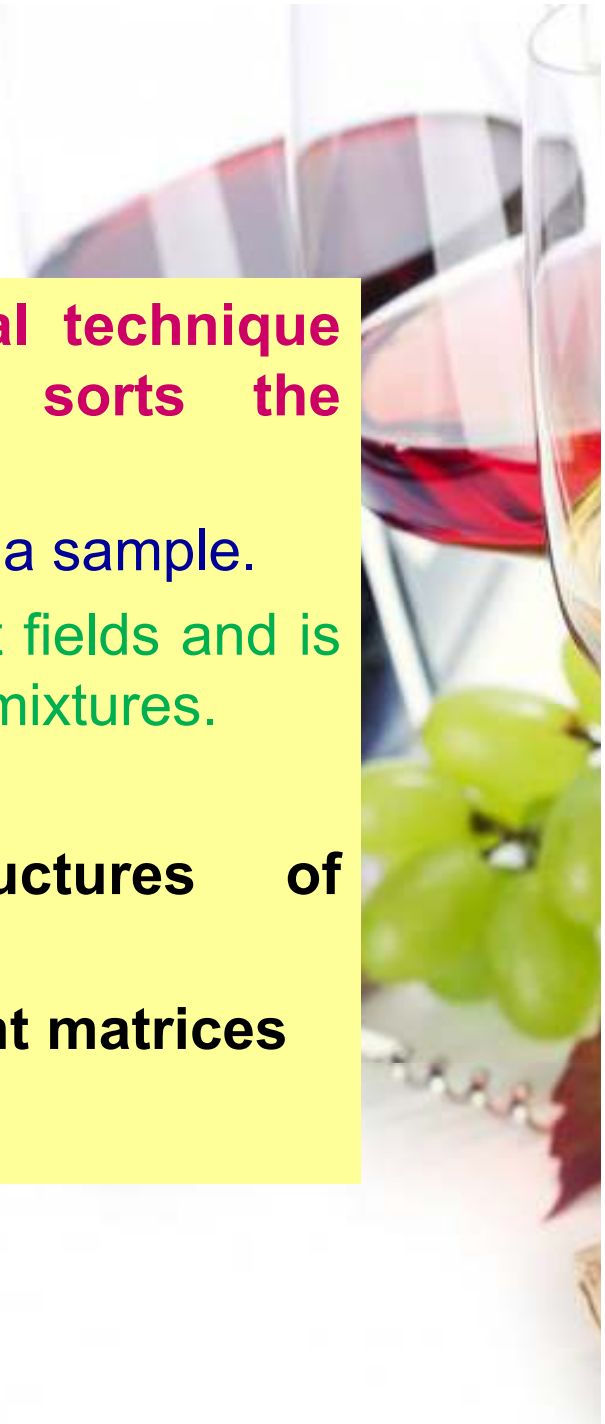


HPLC-DAD analysis of red wine Stanušina



UV-Vis chromatogram of Stanušina wine sample recorded at 530 nm, 324 nm and 280 nm for separation and quantification of anthocyanins, flavan-3-ols/hydroxybenozic acids and hydroxycinnamic acids derivatives, respectively.
Peak identification: gallic acid, (1); (+)-catechin, (2); (-)-epicatechin adducts (3); caftaric acid, (4); coutaric acid, (5); caffeic acid, (6); fertaric acid, (7); delphinidin-3-glucoside, (8); petunidin-3-glucoside, (9); peonidin-3-glucoside, (10); malvidin-3-glucoside, (11); vitisin B, (12); petunidin-(6 acetyl)-3-glucoside, (13); peonidin-(6 acetyl)-3-glucoside, (14); malvidin-(6 acetyl)-3-glucoside, (15); peonidin-coumaroyl-3-glucoside, (16); malvidin-coumaroyl-3-glucoside, (17)

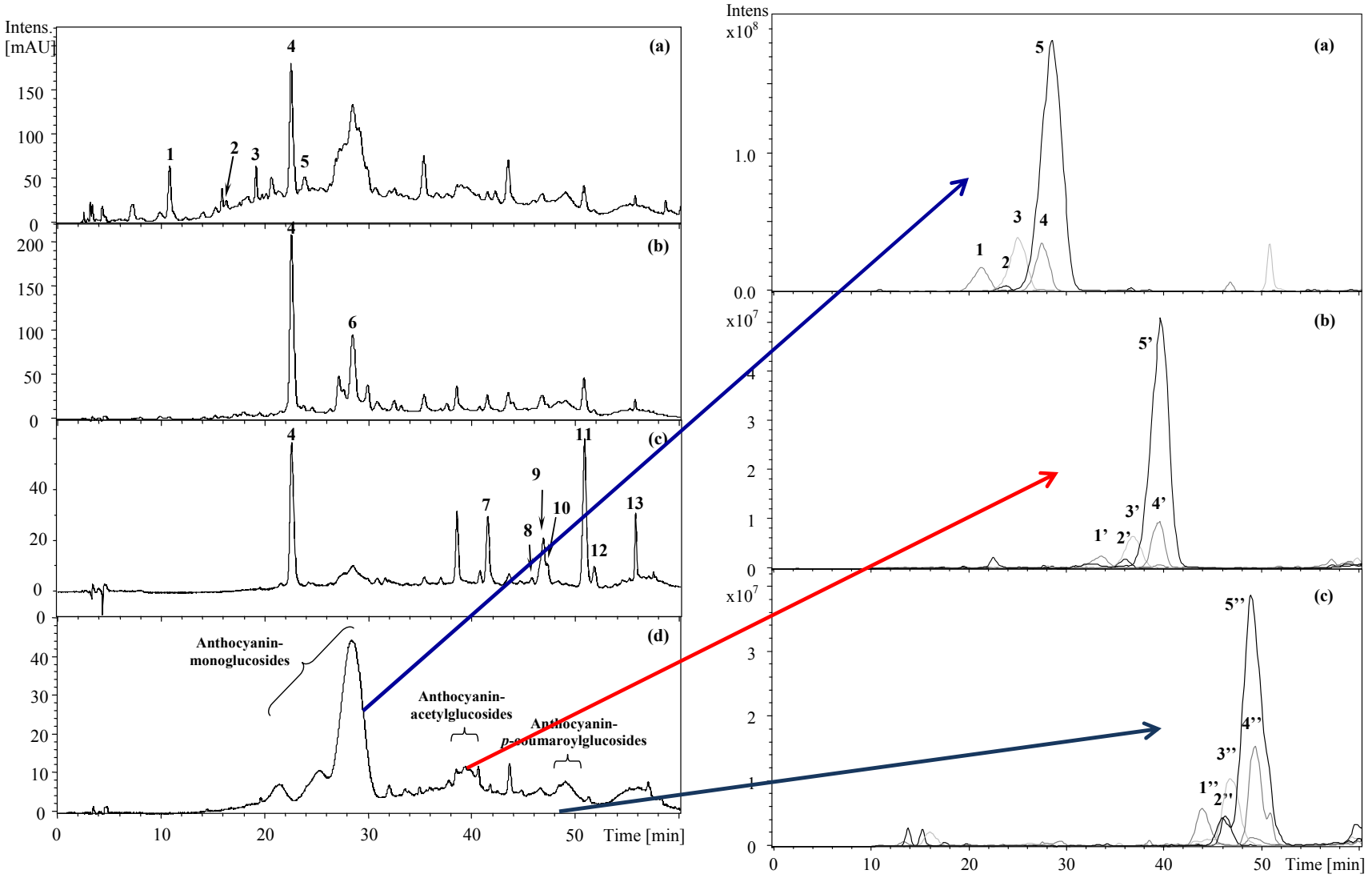
Mass spectrometer



- **Mass spectrometry (MS) is an analytical technique that ionizes chemical species and sorts the ions based on their mass to charge ratio.**
- Mass spectrum measures the masses within a sample.
- Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures.
- **Used for:**
  - **characterization of complex structures of compounds**
  - **detection of new compounds in different matrices**
  - **……**

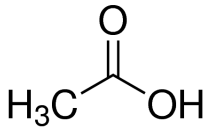
UV and visible chromatograms of polyphenols: (a) 280 nm, (b) 320 nm, (c) 360 nm, (d) 520 nm in Vranec wine

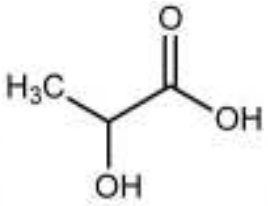
Extracted ion chromatograms at different *m/z* values, which correspond to the M^+ signals of the anthocyanins inVranec wine



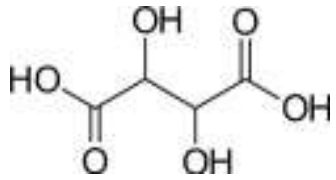
Organic acids in wine

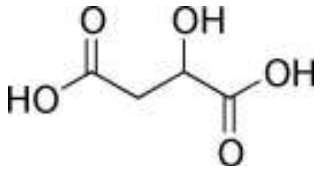
Monoprotic acids:

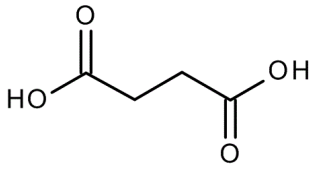
- Acetic acid 

- Lactic acid 

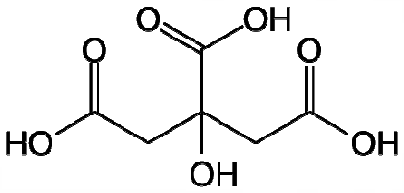
Diprotic acids:

- Tartaric acid 

- Malic acid 

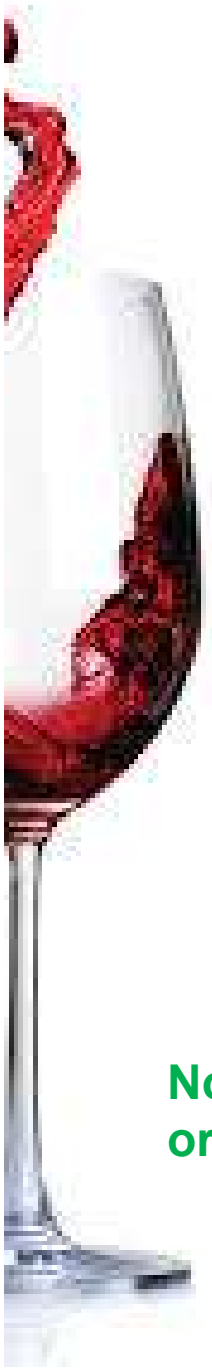
- Succinic acid 

Triprotic acids:

- Citric acid 

❖ ***In grape juices, tartaric, malic and citric acids are the main organic acids.***

❖ ***Acetic, Lactic and Succinic acids are products of fermentation.***



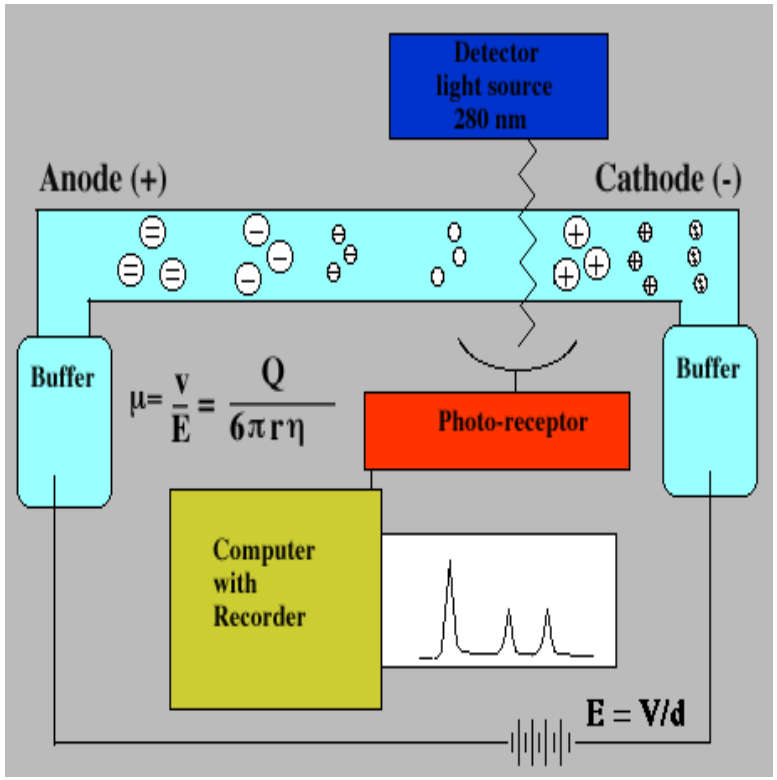
ANALYTICAL TECHNIQUES FOR DETERMINATION OF ORGANIC ACIDS

- ✓ **Chromatographic techniques – HPLC, GC** – Sample preparation necessary!!
- ✓ **Capillary electrophoresis coupled to UV detection -** fast analyses and efficient resolution of the analytes.
- ✓ **Capillary electrophoresis directly coupled to a mass spectrometer (CE-MS) -** higher separation sensitivity, selective mass detection in a single run analysis
- ✓ **Capillary electrophoresis coupled to electrospray ionization mass spectrometer (CE-ESI-MS)**
- ✓ **Capillary electrophoresis coupled to an accurate-mass quadrupole time-of-flight mass spectrometer (QTOF-MS) -** increased sensitivity, provides a high mass accuracy and resolution at high acquisition rates.

No publications where CZE-ESI/QTOF-MS was used for analysis of organic acids in wine samples.

WHAT IS CAPILLARY ELECTROPHORESIS

- In practical terms, a positive (**anode**) and negative (**cathode**) electrode are placed in a solution containing ions.
- Then, when a voltage is applied across the electrodes, solute ions of different charge, i.e. anions (negative) and cations (positive), will move through the solution towards the electrode of opposite charge.
- A photocathode is used to measure the absorbencies of the molecules as they pass through the solution.



- Capillary electrophoresis, then, is the technique of performing electrophoresis in buffer-filled, narrow-bore capillaries, normally from 25 to 100 pm in internal diameter (ID).

CE-ESI/QTOF-MS instrumentation

- **7100 Capillary Electrophoresis (CE) system (Agilent Technologies, Waldbronn, Germany).**
- Detection: 6530 Accurate-Mass Quadrupole Time-of-flight Mass Spectrometer (**QTOF-MS**) coupled to the CE instrument.
- Separation – **Capilary**: 80 cm x 50 µm internal diameter, fused-silica capillary (Polymicro Technologies, Phoenix, USA).
- 1% (*v/v*) solution of formic acid, sheath liquid

Application of the method on organic acids determination in Vranec wines from different regions

The quantitative determination of the organic acids was made by the extracted ion electropherograms for each organic acid. The calculated *m/z* values of the quasi-molecular $[M–H]^-$ ions:

_m/z_ 89.0244 for lactic acid,
_m/z_ 117.0193 for succinic acid,
_m/z_ 133.0142 for malic acid,
_m/z_ 149.0092 for tartaric acid,
_m/z_ 173.0455 for shikimic acid and
_m/z_ 191.0197 for citric acid

CONSLUCIONS

Following techniques allow wine characterization and advanced analyses of its components:

- **GC-MS – volatile compounds**
- **HPLC –DAD – phenolics, organic acids**
- **HPLC-MS – mass characterization**
- ***CE-ESI/QTOF-MS* – organic acids**

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Projects:

- **CEEPUS**, Teaching and learning bioanalysis
- **FP7**
- **Erasmus Plus**

With wine and hope, anything is possible!

Thank you for your attention!