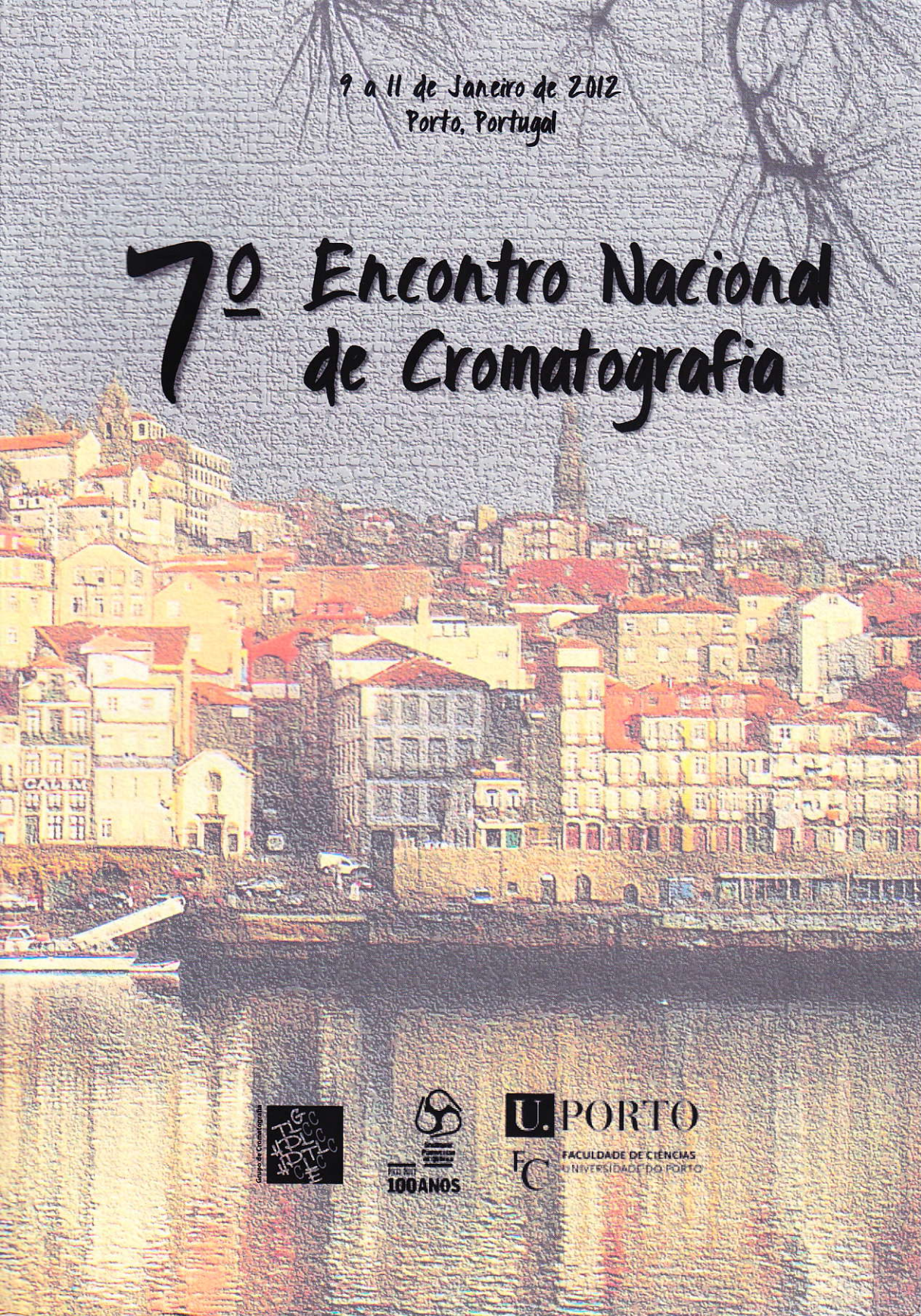
9 a 11 de Janeiro de 2012
Porto, Portugal

7º Encontro Nacional de Cromatografia



Grupo de Cromatografia

100 ANOS

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FACULDADE DE CIÊNCIAS
UNIVERSIDADE DO PORTO

7º Encontro Nacional de Cromatografia

Grupo de Cromatografia
Sociedade Portuguesa de Química

Departamento de Química e Bioquímica
Faculdade de Ciências da Universidade do Porto

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Índice

P87. Monitoring malolactic fermentation using HPLC-UV-RI

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In the malolactic fermentation, which is the second wine fermentation, the malic acid is consumed by lactic acid bacteria to yield lactic acid, resulting in a reduction of the wine fixed acidity. This fermentation can begin during the alcoholic fermentation or after its completion. It must be controlled for improving the organoleptic characteristics and the microbiological stability of wines. If uncontrolled, several unpleasant substances may be formed originating loss of quality of the wine.

The aim of this study was to validate an HPLC method, with UV and RI detectors in series, for monitoring malolactic fermentation to verify its viability as a control method in a fermentation study with an electronic tongue, namely to provide: i) identification of the malolactic fermentation end, by evaluating the malic and lactic acid contents in wine; ii) determination of the levels of different acidic compounds (citric acid, tartaric acid, ascorbic acid and acetic acid), sugars (sucrose, fructose and glucose), glycerol and ethanol along the malolactic fermentation.

This work monitored the malolactic fermentation of a red wine prepared in the Agrarian School of the Politechnic Institute of Bragança, with grapes of the variety "Mourisco Tinto", during five weeks, after completion of the alcoholic fermentation. The HPLC analysis was carried out with a Varian equipment and a Supelcogel C-610H column selected because organic solvents are not required for elution purposes. The above mentioned compounds were analyzed simultaneously for all wine samples collected every 7 days. The chromatographic method showed a good analytical performance, with high sensitivities for each of the analytes and correlation coefficients greater than 0.999. The limits of detection and quantification were low (for example, calculated acid malic LD and LQ values were lower than 0.04 g/L and 0.14 g/L, respectively) and the samples were within the dynamic range defined at work. Sugars were not detected in the wine samples, as expected, demonstrating that the alcoholic fermentation was completed. Moreover, no ascorbic acid was found. However the quantification of citric and lactic acids proved difficult due to interference from unknown compounds.

Monitoring malolactic fermentation using HPLC-UV-RI

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Introduction

Malolactic fermentation → second wine fermentation.

Malic acid consumed → lactic acid bacteria → yield lactic acid
→ reduction of the wine fixed acidity.

Controlled fermentation → improves the organoleptic characteristics
→ microbiological stability of wines.

Uncontrolled fermentation → several unpleasant substances may be formed originating loss of quality of the wine.

Objective

HPLC method validation, with UV and RI detectors in series, for:

1. evaluating the malic and lactic acid contents in wine;
2. identification of the end-point malolactic fermentation;
3. determination of several compounds levels along the malolactic fermentation.
4. to verify its viability as a reference method in a fermentation study with an electronic tongue.

Samples → RED WINE:

→ prepared in the Agrarian School of the IPB;
→ grapes of the variety "Mourisco Tinto";
→ prepared by "PISA" technique;
→ alcoholic fermentation completed;
→ malolactic fermentation monitored in 4 vats;
→ sampling during five weeks, every 7 days;
→ simultaneously UV and RI HPLC analysis – after raw wine sample filtration.

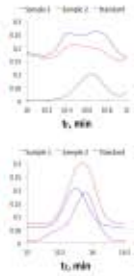
Result

20 Samples – WINE monitorization for 5 weeks:

COMPOUNDS

Citric acid – difficulties in quantifying *1
Tartaric acid – quantified
Ascorbic acid – not detected
Acetic acid – quantified
Malic acid – quantified
Lactic acid – difficulties in quantifying *1
Sucrose – not detected *2
Fructose – not detected *2
Glucose – not detected *2
Glycerol – quantified
Ethanol – quantified

*1) Due to interference from unknown compounds.
*2) The alcoholic fermentation was completed.



HPLC equipment

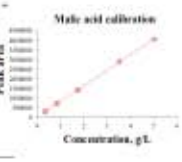
Varian Prostar 220 Pump
Varian 9050 UV (Ultraviolet detector)
Varian RI-4 (refractive index detector)
Rheodyne 7725i manual injector with Loop of 20 μL
Software star chromatography workstation, version 6.4
Jones 7981 chromatography column oven

COLUMN
Supelcogel C-610H de 30cm x 7,8mm DI

GREEN CHEMICAL ANALYSIS

Calibration parameters:

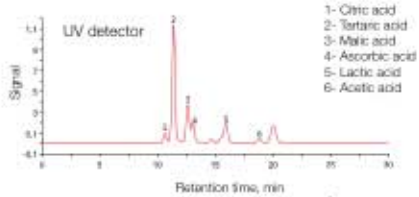
DETECTOR	COMPOUND	SLOPE	INTERCEPT	✔	LOD, g/L	LOQ, g/L
UV	Citric acid	1.01E+6	-1.04E+3	0.99993	0.012	0.041
	Tartaric acid	1.40E+6	8.09E+4	0.99997	0.065	0.22
	Acetic acid	4.71E+5	4.73E+4	0.9998	0.018	0.061
	Malic acid	8.00E+5	1.15E+4	0.99996	0.043	0.14
	Lactic acid	3.81E+5	-2.39E+4	0.9998	0.072	0.24
IR	Glycerol	9.01E+5	1.42E+4	0.9998	0.15	0.49
	Ethanol	7.01E+6	-4.47E+3	0.99998	0.00381	0.002

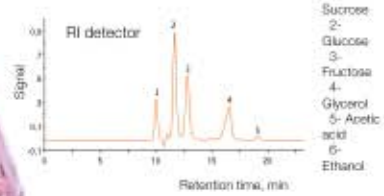


HPLC conditions

Column temperature: 30ºC
Eluent composition: 1% of phosphoric acid aqueous solution
Eluent elution: isocratic
Flux: 0.5 mL/min
Eluent temperature: 40°C

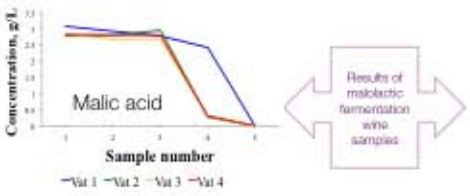
Chromatograms obtained:

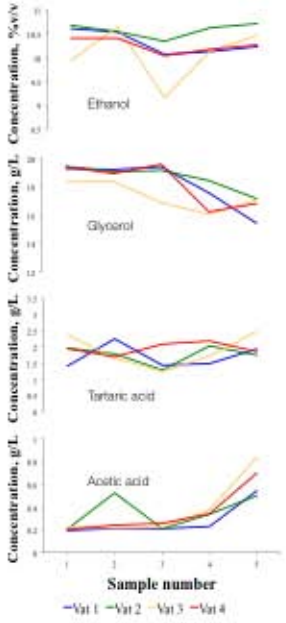




Quality control standard solution:
Acceptable repeatability → Sr%<2.5%
Acceptable intermediate Precision → Sr%<3.5%
Acceptable accuracy → Er%<4.0%

Wine samples:
Acceptable repeatability → Sr%<2.5%
Acceptable intermediate precision → Sr%<3.5%





Conclusions

The overall calibrations and validation study are acceptable.

Analytical results along fermentation showed:

- citric and lactic acids need an alternative peak data treatment.
- tartaric acid and ethanol are approximately constant.
- glycerol acid has a slight decrease at the end of the fermentation.
- acetic acid has a slight increase at the end of the the fermentation.

This analytical method allows to follow the wine malolactic fermentation by analyzing the malic acid concentration.