

9 a 11 de Janeiro de 2012
Porto, Portugal

70^o Encontro Nacional de Cromatografia



7º Encontro Nacional de Cromatografia

Grupo de Cromatografia
Sociedade Portuguesa de Química

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

9 a 11 de Janeiro de 2012

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7º Encontro Nacional de Cromatografia

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7º Encontro Nacional de Cromatografia

Índice

Comprehensive two-dimensional gas chromatography-mass spectrometry (GCxGC-TOFMS) analysis of volatiles in Brazilian <i>Capsicum</i> spp. chili peppers	121
HPLC-DAD-MS analysis of Tempranillo grape seeds from different geographical origin	122
Cafestol, kahweol and related esters profile in espresso coffee	123
On the study of historical Arraiolos tapestries – a chromatographic and spectrometric approach	124
Characterization of phenolic and polysaccharidic extracts of <i>Ganoderma lucidum</i> by chromatographic techniques	125
Antioxidants in <i>Pinus Pinaster</i> and mycorrhizal fungi during the early steps of symbiosis	126
Chromatographic techniques to obtain the biomolecules profile of inedible wild mushrooms with antioxidant value	127
Seasonal variability of essential oils of <i>Lavandula luisieri</i>	128
An overview of the nutrients and non-nutrients present in the wild mushroom species most appreciated in the Northeast of Portugal	129
GC-MS analysis of Portuguese propolis	130
<i>Cytisus multiflorus</i> : phenolic characterization by HPLC-DAD, ESI-MSn and NMR combined methods	131
<i>Mentha aquatica</i> : source of flavanone glycosides	132
Validation of a NP-HPLC-FLD method for tocopherol analysis in fresh lettuce	133
Characterization of volatile compounds of passion fruit species of semi-arid region in the Brazilian Northeast	134
Volatile composition of Portuguese Oak and Portuguese Chestnut wood chips: their relevance for oenological purposes	135
One dimensional and comprehensive two-dimensional gas chromatography analysis of chestnut and oak wood chips with different toasting degrees	136
New RP-HPLC-UV method for the quantification of naphodianthrone from <i>Hypericum</i> species	137
Characterization of <i>Opuntia ficus</i> spp. fractions obtained using Sephadex LH-20.	138
Determination of bisphenol A in mussels using dispersive liquid-liquid microextraction and gas chromatography-mass spectrometry	139
Optimization of in situ derivatization dispersive liquid-liquid microextraction for simultaneous extraction of bisphenol A and bisphenol B in tuna canned followed by gas chromatography-mass spectrometry analysis	140
Development and validation of a simple and sensitive HPLC/FLD method for N-methyl carbamates pesticides in animal tissues based on dispersive extraction by QuEChERS	141

7º Encontro Nacional de Cromatografia

Índice

P72. New strategy to enhance the extraction efficiency of polyphenols from dietary vegetables using a modified QuEChERS combined with UHPLC-PDA system	142
P73. A selective and fast method for the quantitative determination of chloroanisoles in wines using solid phase microextraction and gas chromatography-quadrupole mass spectrometry	143
P74. Development of a QuEChERS-based extraction method for Ochratoxin A analysis in bread by LC-FLD	144
P75. GC-FID and GC-MS as a tool to screen the influence of wood ageing technologies in the brandies odourless and odourant compounds	145
P76. Honey sugars analysis by ion chromatography method with Integrated Pulsed Amperometric Detection (IPAD)	146
P77. Occurrence and bioaccessibility of nitrates in baby foods	147
P78. Avaliação dos níveis de contaminação com patulina em diferentes fases do processamento industrial da fruta	148
P79. Desenvolvimento de um método de análise de melamina em produtos alimentares através de MISPE-HPLC-DAD	149
P80. Quantification of wine ellagitannins by LC-ESI-MS/MS-multiple reaction monitoring	150
P81. Gas-diffusion microextraction: a deeper understanding of the process	151
P82. Determination of free and total diacetyl in wine by HPLC-UV using gas-diffusion microextraction	152
P83. Determination of pesticide residues in grapes and wine using Quechers and liquid chromatography - diode-array detection	153
P84. Recovery and analysis by HPLC-DAD of natural antioxidants from brewer's spent-grain	154
P85. Analysis of acids and sugars in fruit-based drinks by SEC-UV-RI	155
P86. Analysis of honey main sugars by size-exclusion chromatography	156
P87. Monitoring malolactic fermentation using HPLC-UV-RI	157
P88. Determination of chloropropanols in water and food samples using ultrasound-assisted dispersive liquid-liquid microextraction with derivatization and GC-MS detection	158
P89. Arsenic speciation in rice and fish using HPLC-ICP-MS	159
P90. Solid-phase extraction followed by dispersive liquid-liquid microextraction for the sensitive determination of ethylphenols species in wine	160
P91. Fast detection of β -lactam antibiotics in milk by isocratic HPLC elution	161
P92. Chromatographic analysis of macro and micronutrients in the most widely appreciated cultivated mushrooms	162

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

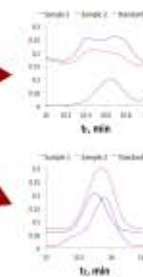
20 Samples – WINE monitoring for 5 weeks:

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 μ L
 Software star chromatography workstation, version 6.4
 Jones 7981 chromatography column oven

COLUMN

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

Calibration parameters:

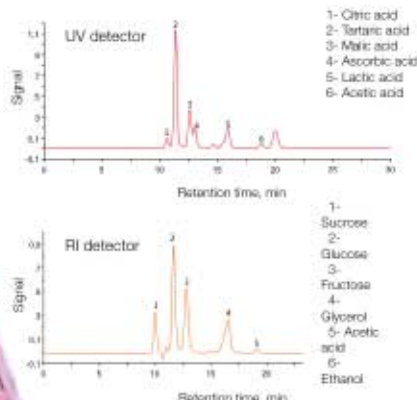
DETECTOR	COMPOUND	SLOPE	INTERCEPT	✓	LOD, g/L	LOQ, g/L	Malic acid calibration
UV	Citric acid	1.01E+6	-1.04E+3	0.99993	0.012	0.041	
	Tartaric acid	1.40E+6	8.09E+4	0.99997	0.005	0.22	
	Acetic acid	4.71E+5	4.73E+4	0.9998	0.018	0.061	
	Malic acid	8.00E+5	1.15E+4	0.99998	0.043	0.24	
	Lactic acid	3.01E+5	-2.39E+4	0.9998	0.072	0.24	
	RI	Glycerol	9.01E+5	1.42E+4	0.9998	0.15	
	Ethanol	7.01E+6	-4.47E+3	0.99998	0.00381	0.002	

GREEN CHEMICAL ANALYSIS

HPLC conditions

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

Chromatograms obtained:

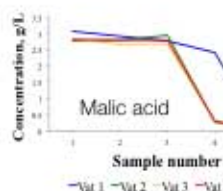


Quality control standard solution:

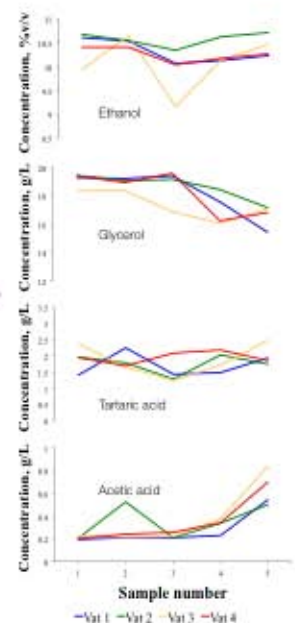
- Acceptable repeatability → Sr% < 2.5%
- Acceptable intermediate Precision → Sr% < 3.5%
- Acceptable accuracy → Er% < 4.0%

Wine samples:

- Acceptable repeatability → Sr% < 2.5%
- Acceptable intermediate precision → Sr% < 3.5%



Results of malolactic fermentation wine samples



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 fermentation.

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

