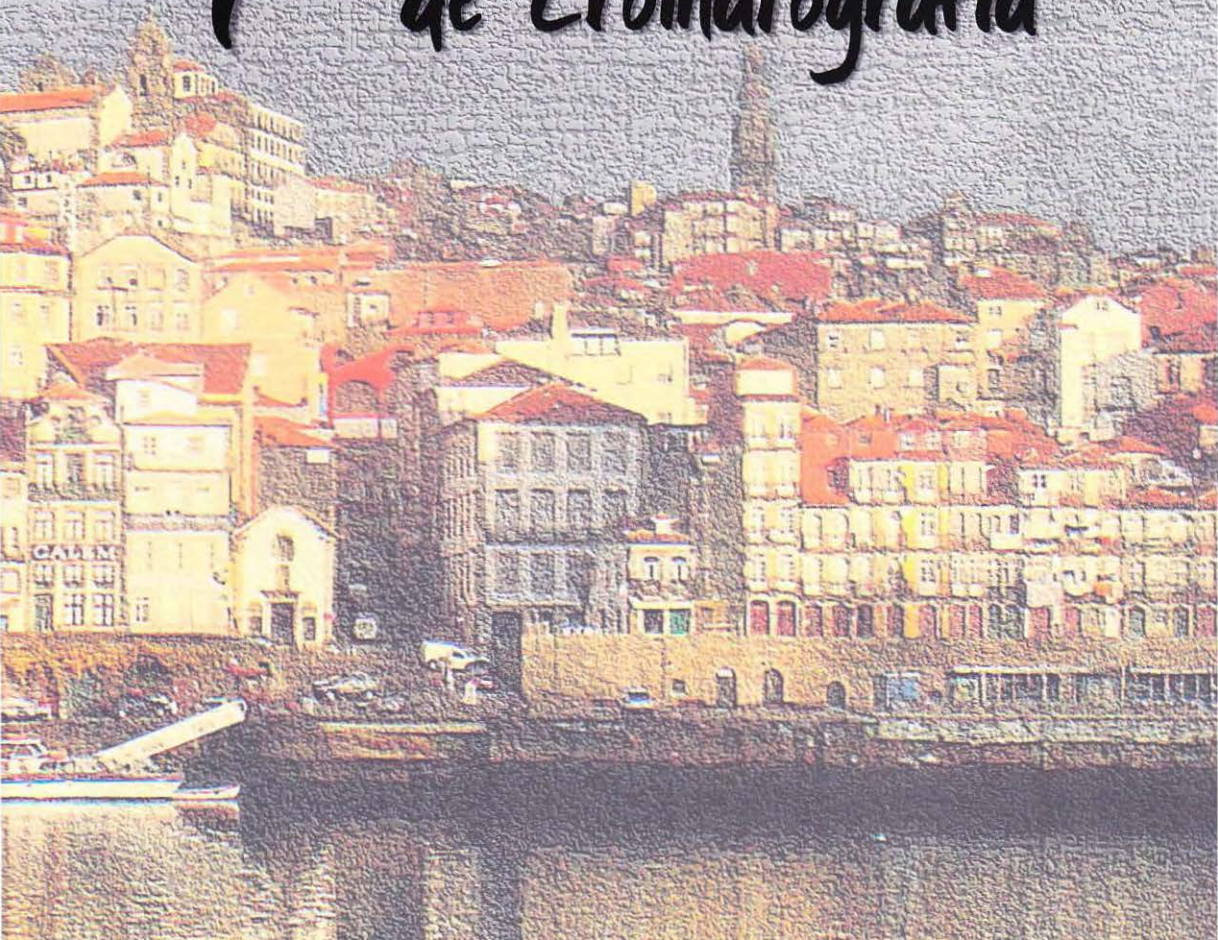


9 a 11 de Janeiro de 2012  
Porto, Portugal

# 70<sup>o</sup> 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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## **P86. Analysis of honey main sugars by size-exclusion chromatography**

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The main constituents of honey are sugars, mainly the monosaccharides fructose and glucose (reducing sugars), which account for 80% of the total mass, while the disaccharides (especially sucrose and maltose) represent 10%. In general, fructose predominates (giving a sweeter taste to honey than sucrose), although, in some honeys, glucose (that assigns a less sweet taste than sucrose but more than maltose) may be the main sugar. The fructose/glucose mass ratio is a parameter that allows the identification of possible adulterations of honey, by signaling abnormal levels of glucose due to the addition of commercial glucose or syrups. It also allows assessing the crystallization trend of the honey, although the glucose/water ratio provides a more accurate indication of this behavior. Therefore, for marketing purposes, the quantification of glucose and fructose in honey is a key factor for establishing its quality. This work reports the validation of a method for determination of honey sugars by SEC to be used as control in the study of their determination with an electronic tongue.

A set of honey samples was provided by the Portuguese National Federation of Beekeepers to obtain a national sampling which showed the natural composition variability of Portuguese honeys. The analysis were carried out with HPLC equipment from Varian and a Supelcogel C-610H column, selected because the elution does not require organic solvents. The target compounds to analyze were glucose and fructose for which well separated peaks were found in the chromatograms, which included other two, for a mix of polysaccharides and unknown substance(s).

The results of a thorough validation showed that the method can be employed for quantification of these two sugars in honey samples.

# ANALYSIS OF HONEY MAIN SUGARS BY SIZE-EXCLUSION CHROMATOGRAPHY

Mara E.B.C. Sousa<sup>a,\*</sup>, António M. Peres<sup>a,b</sup>, Luís G. Dias<sup>a</sup>, Jorge Sá Morais<sup>a</sup>,  
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## Introduction

Main constituents of honey are sugars:

- = 80% fructose and glucose;
- 10% disaccharides (especially sucrose and maltose).

Fructose predominates in honey  
In some, glucose may be the main sugar.

Fructose/glucose mass ratio:

- identification of possible adulterations of honey;
- >1.3 will crystallize slowly or remain liquid.

## Objectives

HPLC method validation for honey sugars determination by SEC (size-exclusion chromatography)

Reference method in the study of their determination with an electronic tongue.

## Samples

Honeys provided by FNAP - Portuguese Federation of Beekeepers Portugal from different Portugal regions.  
Samples preparation - DILUTION.

## HPLC equipment

Varian Prostar 220 Pump  
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

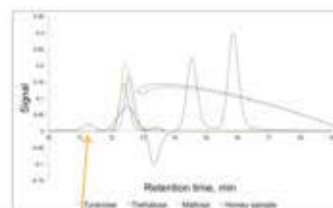
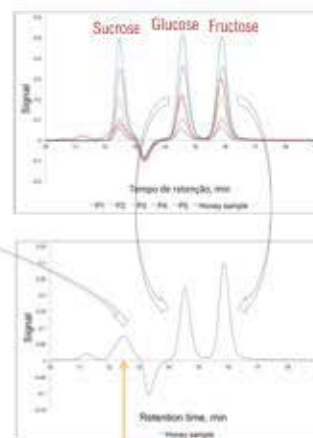
## HPLC conditions

Column temperature: 30°C  
Eluent composition: 1% of phosphoric acid aqueous solution  
Eluent elution: isocratic  
Flux: 0.4 mL/min  
Eluent temperature: 40°C

## Results

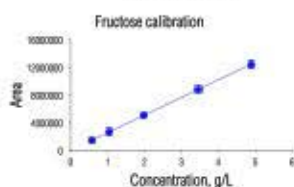
1. Compound identification - retention time.
2. Calibration - glucose and fructose.
3. Study of ACCURACY and PRECISION:
  - 2 quality control solutions;
  - 3 honey samples (different colors).
4. Ten different honeys analysed.

1



Disaccharides + Trisaccharides + ?

Disaccharides + Trisaccharides + ?



2

### CALIBRATION PARAMETERS

Compound	Cmin, g/L	Cmax, g/L	Slope	Intercept	Correlation	DL, g/L	QL, g/L
Glucose	0.901	4.968	2952412.3	37902.6	0.99995	0.061	0.154
Fructose	0.588	4.886	2950202.6	22631.8	0.99995	0.061	0.155

Cmin - minimum concentration  
Cmax - maximum concentration  
DL - detection limit  
QL - quantification limit

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### Quality control solutions repeatability

Solution	Compound	Expected concentration (g/L)	Experimental concentration (g/L)	Standard deviation (s) (g/L)	Relative standard deviation (sr%)	Relative error (Er%)
QCS1	Glucose	2.11	2.19	0.01	0.7	1.8
	Fructose	3.21	3.27	0.01	0.4	1.9
QCS2	Glucose	0.745	0.75	0.01	1.9	0.2
	Fructose	0.731	0.74	0.01	1.6	0.8

QCS - quality control solutions

### Quality control solutions intermediate precision

Solution	Compound	Expected concentration (g/L)	Experimental concentration (g/L)	Standard deviation (s) (g/L)	Relative standard deviation (sr%)	Relative error (Er%)
QCS1	Glucose	2.11	2.1	0.1	4.9	0.7
	Fructose	3.21	3.2	0.1	3.4	0.7
QCS2	Glucose	0.75	0.74	0.01	1.7	0.08
	Fructose	0.73	0.74	0.01	1.7	0.8

QCS - quality control solutions

### Samples repeatability

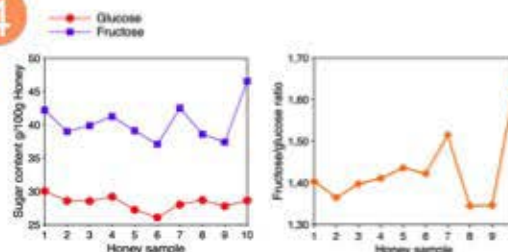
Solution	Compound	Experimental concentration (g/100g Honey)	Standard deviation (s) (g/100g Honey)	Relative standard deviation (sr%)
Honey 1	Glucose	25.6	0.2	0.7
	Fructose	39.0	0.2	0.4
Honey 2	Glucose	25.22	0.09	0.3
	Fructose	42.81	0.09	0.2
Honey 3	Glucose	27.7	0.1	0.4
	Fructose	39.20	0.09	0.2

### Samples intermediate precision

Solution	Compound	Experimental concentration (g/100g Honey)	Standard deviation (s) (g/100g Honey)	Relative standard deviation (sr%)
Honey 1	Glucose	25	1	5.0
	Fructose	39	1	3.4
Honey 2	Glucose	25.6	0.3	1.0
	Fructose	43.3	0.4	1.0
Honey 3	Glucose	28.5	0.6	2.1
	Fructose	39.7	0.3	0.7

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10 HONEY SAMPLES



## Conclusions

- The overall calibrations are acceptable → Results of accuracy and precision are acceptable.
- The method can be employed for quantification of these two sugars in honey samples.
- Fructose in honey always greater than glucose → Fructose/glucose mass ratio in honey higher than 1.34.
- Honeys will crystallize slowly or remain liquid.

