Optimized determination of honey carbohydrates by HPAEC-PAD

Ivan Obreshkov, Dorit Franz, Ingo Schellenberg

Abstract: *A novel HPAEC-PAD method was developed. The determination of eight carbohydrates in a single run within six minutes at room temperature (20 ± 2 °C) was demonstrated. The carbohydrates were isocratically eluted with 100 mM NaOH using CarboPac PA-100 column (4 × 250 mm). The flow-rate was 0.9 mL.min^{-1} and the sample injection volume was 25 µL. Since each resolution factor (Rs) was above 1.1, the carbohydrates were fully separated. The elution order was trehalose, rhamnose, arabinose, glucose, fructose, saccharose, raffinose, and melezitoze.*

Key words: *carbohydrates, honey, HPAEC-PAD.*

INTRODUCTION

Honey production in 2011 was 1636 kilotons. The EU is the second largest producer (13.3 % of the world production), after China (27.3 %). The honey sugar analysis proves the natural origin of the product and excludes products that can not be defined as honeys. Some of the most important characteristics of honey include the carbohydrate content, particularly the glucose and the fructose content, and their total quantity. Some authors demonstrate also that the ratio between glucose and fructose is another parameter of interest. The HPAEC-PAD technique is the one for accurate determination of the carbohydrates acting as weak acids [2, 3, 4, 5, 9, 10, 11, 12]. Among the basic characteristics of a method are concentration of eluents, the flow-rate, elution regime and temperature. All of them are influencing the run time of the analyses. A method for honey analyses was developed and validated allowing the determination of nine carbohydrates within 33 minutes [9,10]. The times reduction results in higher number of processed samples and in combination with reduced quantity of eluents could achieve higher efficiency in terms of lower costs.

The aim of the present study was to develop a HPAEC-PAD method in order to achieve shorter analytical time.

EXPERIMENTAL, RESULTS AND DISCUSSION

Chemicals

The following analytical sugar standards were used: D(+)-glucose, D(-)-fructose and D(+)-maltose monohydrate (Merck, Darmstadt, Germany); D(+)-trehalose dehydrate, L(+)-rhamnose monohydrate, D(+)-melezitose monohydrate and D(+)-raffinose pentahydrate (Carl Roth GmBH, Karlsruhe, Germany); L(+)-arabinose (Fritz Leidholdt-Biochemie, Germany); D(+)-sucrose (Fluka, Ronkonkoma, USA). Fifty percent (w/w) sodium hydroxide solution was obtained from J.T. Baker (Deventer, Netherlands).

Data processing

The eluted carbohydrates were plotted with Chromeleon Client 6.60 SP1a Build 1449 (Dionex) and the data were processed with Origin 8.0. All analyses were carried out in triplicates.

Chromatography

The carbohydrate separation was performed on a Dionex IC system, model DX-500 (Sunnyvale, CA, USA), equipped with a GP-40 gradient pump, AS 40 autosampler, LC20 chromatography enclosure and ED40 pulsed amperometric detector with a gold working electrode. The pulse potentials on the ED40 detector were: t = 0, E = 0.05 V; t = 0.20 s, E = 0.05 V (start); t = 0.40 s, E = 0.05 (end); t = 0.41 s, E = 0.75 V; t = 0.60 s, E = 0.75; t = 0.61 s, E = -0.15 V; t = 1.00 s, E = -0.15 V [1]. After injection of a filtered sample (25 µL) into a CarboPac PA-100 column (4 × 250 mm) (Dionex Corp., USA), the carbohydrates were isocratically eluted with a mobile phase of 100 mM NaOH at flow-rate of 0.9 mL.min^{-1}

for 6 minutes run time. The carbohydrates were identified by comparison with the retention times of external standards.

Eight carbohydrates were eluted during the six minutes analytical time. The maltose was not eluted within that period. In Table 1, the peak identification data are shown.

Table 1. Peak identification (n=21)

	Peak		Statistical Data	
Standard	RT*	SD**	R^2	Grubbs' Outlier
Trehalose	2.06	0.01	0.9952	No
Rhamnose	2.43	0.01	0.9980	No
Arabinose	2.66	0.02	0.9988	No
Glucose	2.96	0.03	0.9982	No
Fructose	3.25	0.03	0.9990	No
Saccharose	4.06	0.07	0.9996	No
Raffinose	5.12	0.11	0.9974	No
Melezitose	5.42	0.11	0.9973	No

**RT – Retention Time; **SD – standard deviation*

The elution order was trehalose, rhamnose, arabinose, glucose, fructose, saccharose, raffinose, melezitoze. The theoretical calibration mathematical models were characterized with high coefficients of determination (R^2) and low standard errors. The coefficients of determination varied within the range of 0.9952 and 0.9996, for trehalose and saccharose, respectively. No Grubbs outliers were detected [6].

Table 2 demonstrates the achieved resolution factors of the peaks.

Table 2. Resolution Factors (n=21)

Peaks	*Rs*	SD
Trehalose – Rhamnose	3.0	0.2
Rhamnose – Arabinose	1.8	0.1
Arabinose – Glucose	2.1	0.1
Glucose – Fructose	1.8	0.1
Fructose – Saccharose	4.1	0.4
Saccharose – Raffinose	4.2	0.4
Raffinose – Melezitose	1.1	0.1

Rs – resolution factors; SD – standard deviation

The obtained results showed that the separation of all eight carbohydrates was achieved at acceptable level. The maximum peak resolutions reached 4.2. The calibration procedure at seven concentrations was applied. Except the saccharose, all other carbohydrates allowed linear calibration providing information on LOD and LOQ values as follows: trehalose (LOD = 14.05 ppm; LOQ = 47.17 ppm), rhamnose (LOD = 7.85 ppm; LOQ = 24.83 ppm), arabinose (LOD = 6.21 ppm; LOQ = 20.02 ppm), glucose (LOD = 7.48 ppm; LOQ = 23.76 ppm), fructose (LOD = 5.56 ppm; LOQ = 18.08 ppm), raffinose (LOD = 10.43 ppm; LOQ = 34.40 ppm), and melezitoze (LOD = 8.46 ppm; LOQ = 26.32 ppm).

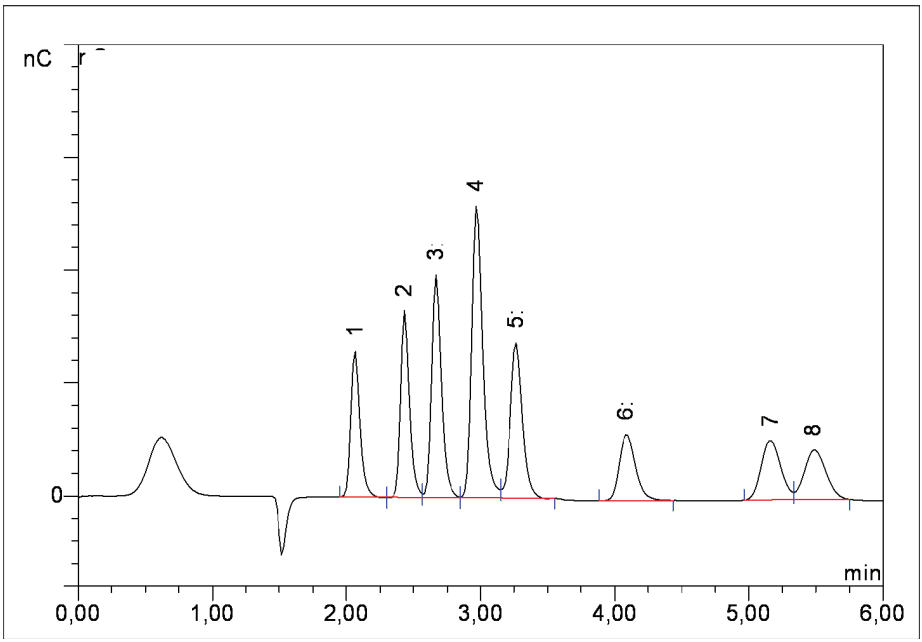

Fig. 1. HPLC-PAD chromatogram for the separation of the carbohydrates: 1 – trehalose; 2 – rhamnose; 3 – arabinose; 4 – glucose; 5 – fructose; 6 – saccharose; 7 – raffinose; 8 – melezitoze.

The shorter duration of the analyses reduced the quantity of the eluent from 16.5 mL to 5.4 mL pro analytical run.

CONCLUSION

The developed method simultaneously identified eight carbohydrates within six minutes in a single run. The shorter time for analyses favours the higher number of samples that could be processed for a period of time as well as lower analytical costs. The elution order was: trehalose, rhamnose, arabinose, glucose, fructose, saccharose, raffinose, melezitoze.

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This paper has been reviewed