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Simple column-switching ion chromatography method for determining eight monosaccharides and oligosaccharides in honeydew and nectar

Chengzhu Ni [a,c], Binhe Zhu [a,c], Nani Wang [a,d,*], Muhua Wang [b], Suqing Chen [e], Jiajie Zhang [a,c], Yan Zhu [a,c,*]

[a] Department of Chemistry, Xixi Campus, Zhejiang University, Hangzhou 310007, China
[b] Department of Chemistry, Lishui University, Lishui 323000, China
[c] Key Laboratory of Health Risk Appraisal for Trace Toxic Chemicals of Zhejiang Province, Hangzhou 310007, China
[d] Department of Medicine, Zhejiang Academy of Traditional Chinese Medicine, Hangzhou 310007, China
[e] School of Pharmaceutical and Chemical Engineering, Taizhou University, Taizhou 317000,China

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ABSTRACT

Honeydew is excreted by aphids as a sweet waste and nectar is floral honey. Honeydew and nectar are complicated samples which consist of various sugars and amino acids. In this work, a simple ion chromatography with column-switching method was developed for the simultaneous analysis of 8 monosaccharides and oligosaccharides in honeydew and nectar. A reversed-phase column was used as a pretreatment column to eliminate organics on-line and sugars were eluted from a collection loop to analytical column by using column-switching technique. This method showed good linearity ($r \geqslant 0.9994$) and afforded low limits of detection ranging from 1.55 to 10.17 μg L^{-1} for all the analytes. Recoveries ranged from 95% to 105% and repeatability results were acceptable with relative standard deviation of less than 3.21% ($n = 6$). This method was successfully applied to quantification of these sugars in honeydew and nectar. These results showed honeydew had much more oligosaccharides than nectar.

1. Introduction

Honeydew is excreted by aphids as a sweet waste which consists mainly of various sugars and amino acids (Fischer, Völkl, & Hoffmann, 2005). Sugar compositions of honeydew vary significantly between aphid species but may also vary within a particular aphid species in an age-specific pattern or when feeding on different host plants (Fischer et al., 2005). Besides, there is a relationship between ant tending and honeydew sugar composition (Fischer & Shingleton, 2001). Nectar is another important element in agriculture. Nectar chemistry, including the sugar proportion, may differ among populations, individuals, cultivars or subspecies of the same species (Agostini, Sazima, & Galetto, 2011). In some regions, especially in Central and Eastern Europe, honeydew honey is highly valued in public as it has more benefits on human health than floral (nectar) honey (Simova, Atanassov, Shishiniova, & Bankova, 2012). Moreover, honeydew is appreciably higher in oligosaccharides than the nectar (Simova et al., 2012).

Till now, several methods regarding sugar analysis have been investigated. These include a variety of chromatographic methods such as high performance liquid chromatography (HPLC) (Földházi, 1994; Swallow & Low, 1990), gas chromatography (GC) (Low & Sporns, 1988), and gas chromatography–mass spectrometry (GC–MS) (Molnár-Perl, 1999). All of these methods can be utilized to separate and quantified the major sugars in honey. However, most of them have occasional disadvantages. For example, some chromatography methods require derivatization, such as GC, GC–MS, HPLC-diode array detector (HPLC-DAD) and HPLC-fluorescence detector (HPLC-FLD) (Pico, Martínez, Martín, & Gómez, 2015). HPLC–mass spectrometry (HPLC–MS) is expensive, and HPLC-refractive index detector (HPLC-RI) does not allow the use of a gradient and has low sensitivity (Molnár-Perl, 1999).

Ion chromatography (IC) is a special kind of HPLC which is widely used to analyze cations, anions, and some important biological compounds such as sugars, amino acids, peptides and proteins. For example, IC-pulsed amperometric detection (IC-PAD) is one of the most useful techniques for determination of oligosaccharide (Ouchemoukh, Schweitzer, Bey, & Djoudad-Kadji, 2010; Steppuhn & Wäckers, 2004). PAD proved to be superior to RI detection and evaporative light scattering detection (ELSD) in aspect of selectivity, sensitivity and having possibility of gradient elution (Molnár-Perl, 1999). However, honeydew and nectar contain sugars as well as vitamins, amino acids, proteins. Organics in samples not only lead to damage the IC columns, but also may make erroneous results. Thus, it is important to remove organics from sample before analysis. The conventional sample preparation method is

* Corresponding authors at: Department of Chemistry, Xixi Campus, Zhejiang University, Tianmushan Road 148, 310007 Hangzhou, China.
E-mail addresses: wnn8511@163.com (N. Wang), zhuyan@zju.edu.cn (Y. Zhu).

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Table 1
System operation procedure.

Procedure	Time (min)	Mobile phase of analytical column	Mobile phase of pretreatment column	Valve 1	Valve 2
1. Sampling		35 mmol L^{-1} NaOH	35 mmol L^{-1} NaOH	Load	Inject
2. Sample pretreatment	0–3.0	35 mmol L^{-1} NaOH	35 mmol L^{-1} NaOH	Inject	Load
3. Sugars analyzing and rinsing of NG1	3.0–18.0	35 mmol L^{-1} NaOH	Acetonitrile	Load	Inject
	18.0–23.0	35–165 mmol L^{-1} NaOH	Acetonitrile	Load	Inject
4. Sugar analyzing and achieving the equilibrium state of NG1	23.0–32.0	165 mmol L^{-1} NaOH	35 mmol L^{-1} NaOH	Load	Inject
5. System achieving the equilibrium state	32.0–35.0	35 mmol L^{-1} NaOH	35 mmol L^{-1} NaOH	Load	Inject

using a reverse-phase pretreatment column, such as Thermo Fisher OnGuard II RP column. Although these pretreatment columns can be reused 5–10 times, the regeneration processes are time-consuming due to manual operations. Therefore, on-line pretreatment method is proposed.

As an effective on-line sample pretreatment method, column-switching technique has been widely used in ion chromatographic system (Huang, Subhani, Zhu, Guo, & Zhu, 2013; Zhu et al., 2013). For example, Yang et al. (2013) proposed a simple IC column-switching method for detection of hexavalent chromium in *Colla corii asini*. Sample solution with target ions were eluted from a collection loop to analytical columns, with on-line matrix elimination. Zhong, Zhou, Zeng, Ye, and Zhu (2011) used an IC system coupled with on-line column-switching technique to determine anions in organic chemicals of high purity. Zhu et al. (2013) proposed the column-switching IC-PAD method for simultaneous determination of glucose, D-gluconic acid, 2-keto-D-gluconic acid and 5-keto-D-gluconic acid.

In this work, a column-switching IC method was established for the analysis of sugars in honeydew and nectar. The samples could be analyzed after on-line elimination of the matrices. It has potential to provide a fast, convenient, and practical approach for analysis of sugars in biological samples.

2. Experimental

2.1. Equipment

An ICS 3000 IC system (Thermo-Fisher Scientific Waltham, MA, USA) was employed for all the chromatographic separations, which was equipped with a quaternary pump, a column heater, two six-port valves (P/N 061961, Rheodyne, Cotati, CA, USA). The electrochemical detector (ED50 electrochemical detector, Thermo-Fisher Scientific Waltham, MA, USA) was equipped with a gold working electrode, an pH/Ag/AgCl composite reference electrode. A quaternary pump (Ultimate 3000, Thermo-Fisher Scientific Waltham, MA, USA) was used to supply the pretreatment solution and washing solution for the pretreatment column. An UV detector (Ultimate 3000, Thermo-Fisher Scientific Waltham, MA, USA) was applied in validation experiment. All columns used in this study were manufactured by Thermo-Fisher Scientific. CarboPac PA 10 guard column (50 mm × 4 mm) and CarboPac PA 10 (250 mm × 4 mm) separation column were used for the sugar separation. An IonPac NG1 guard column (50 mm × 4 mm) was used as a pretreatment column. 25 μL of prepared sample was injected into the loop of the chromatograph and a 2 mL polyether ether ketone tube used as a collection loop. Polyether ether ketone (PEEK) tubes with the lengths as short as possible were used to connect all chromatographic hardware. The eluent flow rate was 1.0 mL min^{-1}. Data were collected with Chromeleon 6.80 chromatogram workstation (Thermo-Fisher Scientific Waltham, MA, USA).

The cell of the pulsed amperometric detector was kept at the temperature of 35 °C. The time and voltage parameters for the pulsed amperometric detector were +0.10 V from 0.00 to 0.40 s, −2.00 V from 0.41 to 0.42 s, +0.60 V at 0.43 s, −0.10 V from 0.44 to 0.50 s, with current integrated between 0.20 s and 0.40 s for detection using the Ag/AgCl reference electrode mode. The separation is achieved with a gradient of two mobile phases. Phase A was ultra-pure water and phase B was 0.25 mol L^{-1} NaOH (HPLC grade, Fischer-Scientific, USA). The system was operated

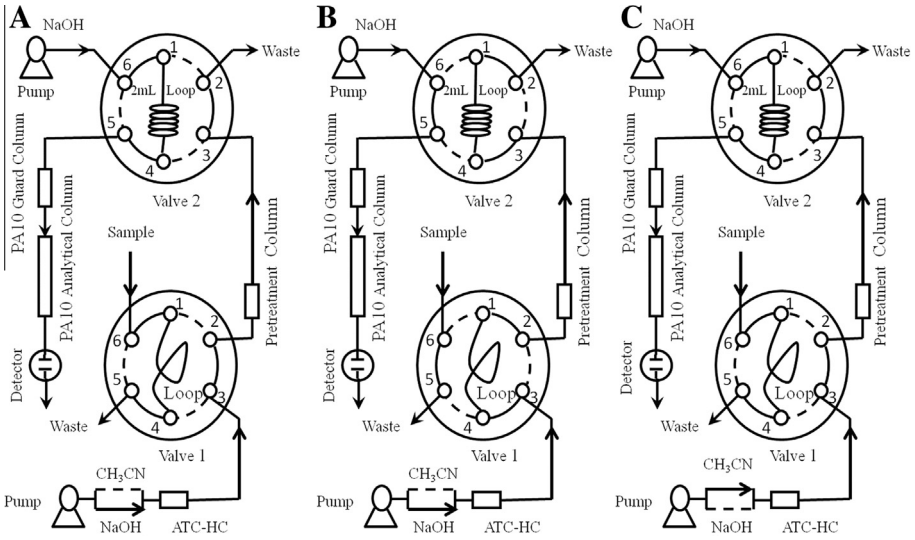

Fig. 1. Chromatographic instrument configuration for the column-switching system. (A) filling the sample loop; (B) on-line sample pretreatment; (C) analyzing the sugars and washing the pretreatment column.