Food Chemistry 180 (2015) 265–271

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Improved evaporative light scattering detection for carbohydrate analysis

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ARTICLE INFO

Article history:
Received 21 October 2014
Received in revised form 15 December 2014
Accepted 7 February 2015
Available online 14 February 2015

Keywords:
ELSD
HPSEC
Carbohydrate
Polysaccharide
Oligosaccharide
RSM

ABSTRACT

Optimization and validation of evaporative light scattering detector (ELSD), aided by response surface methodology (RSM), has been developed for the liquid chromatography analysis of a wide molecular weight (MW) range of carbohydrates, including polysaccharides and oligosaccharides. Optimal experimental parameters for the ELSD detection were: 88.8 °C evaporator temperature, 77.9 °C nebulizer temperature and 1.1 standard litres per minute nitrogen flow rate. Optimal ELSD detection, used together with high performance size exclusion chromatography (HPSEC) of carbohydrates, gave a linear range from 250 to 1000 mg L^{-1} ($R^2 > 0.998$), with limits of detection and quantitation of 4.83–11.67 and 16.11–38.91 mg L^{-1}, respectively. Relative standard deviation was lower than 1.8% for intra-day and inter-day repeatability for apple pectin, inulin, verbascose, stachyose and raffinose. Recovery ranged from 103.7% to 118.3% for fructo-oligosaccharides, α-galacto-oligosaccharides and disaccharides. Optimized and validated ELSD detection is proposed for the analysis of high- to low-MW carbohydrates with high sensitivity, precision and accuracy.

1. Introduction

Refractive index detector (RID) measurement is the most popular detector for the analysis of carbohydrates by high performance liquid chromatography (HPLC). RID is a universal and non destructive detector which response is proportional to compound concentration within a wide range (Raessler, 2011). However, this detector has some disadvantages, such as its relatively low sensitivity, dependence of response on temperature and flow rate of eluent, as well as its incompatibility with gradient elution (Gómez-Ordóñez, Jiménez-Escrig, & Rupérez, 2012; Nogueira, Silva, Ferreira, & Trugo, 2005). Evaporative light scattering is an increasingly used mass detector for the HPLC analysis of carbohydrates. It is a semi-universal detector based on the detection of solute molecules by light scattering after nebulization and evaporation of the mobile phase; so ELSD is suitable to detect compounds with low volatility during the vaporization process, such as carbohydrates. As an advantage against RID, the ELSD has a lower limit of detection for carbohydrates and it allows to work with gradient elution (Lafosse & Herbreteau, 2002; Nogueira et al., 2005). The nebulization of the chromatographic effluent into gas stream allows in the vaporization step to generate aerosol droplets that scattered the light and determine the signal intensity of ELSD (Lafosse & Herbreteau, 2002). However, nebulization can have an influence on the aerosol droplet size (ranged from 4 to 40 μm) which, in turn, can modify the detector response (Lafosse & Herbreteau, 2002; Ma, Sun, Chen, Zhang, & Zhu, 2014). Consequently, there is a need to define the optimal conditions for ELSD.

In this regard, Box–Behnken design has been reported as an alternative for the optimization of analytical methods by response surface methodology (Ferreira et al., 2007), although this methodology has not been applied previously to optimize the parameters of ELSD (temperature of evaporator and nebulizer, gas flow rate) for carbohydrate analysis.

Until now ELSD detection in food samples has been used for the analyses of sugars (Liu et al., 2012; Ma et al., 2014; Nogueira et al., 2005; Shanmugavelan et al., 2013), oligosaccharides (Westphal, Schols, Voragen, & Gruppen, 2010), malto-oligosaccharides (Zhou et al., 2014), fructo-oligosaccharides (Downes & Terry, 2010) and polysaccharides, such as native starches (Kärkkäinen, Lappalainen, Joensuu, & Lajunen, 2011). However, to the best of our knowledge, currently no HPLC methods have been fully developed for the analysis of low- (simple sugars), medium- (oligosaccharides) and high-molecular weight carbohydrates (polysaccharides) using

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http://dx.doi.org/10.1016/j.foodchem.2015.02.039
0308-8146/

evaporative light scattering detection. Therefore, our main aim was optimize and validate the ELSD-assisted by RSM as a tool – for the chromatographic analysis of a wide molecular weight range of carbohydrates with high sensitivity, precision and good accuracy, including monosaccharides, disaccharides, oligosaccharides (such as fructo- and α-galacto-oligosaccharides) and polysaccharides.

2. Materials and methods

2.1. Reagents

Carbohydrate standards of different molecular weight were used for calibration. Pullullan Shodex standards [P-82 kit; Cat. #: WAT034207: P-100 (MW = 112 kDa) and P-50 (MW = 47.3 kDa)] were obtained from Waters Chromatography (Madrid, Spain). Dextran standards: blue dextran T-2000 (Cat. #: 17-0330-01; MW = 2000 kDa), T-500 (Cat. #: 17-0320-01; MW = 500 kDa), T-70 (Cat. #: 17-0280-01; MW = 70 kDa) and T-10 (Cat. #: 17-0250-01; MW = 10 kDa) were obtained from Pharmacia Biotech Europe GmbH (Barcelona, Spain). D(+)-glucose (Cat. #: 108337; MW = 180 Da) from Merck (Darmstadt, Germany) and maltodextrin (Cat. #: 419699; 16.5-19.5 glucose equivalents) from Aldrich (Alcobendas, Madrid, Spain) were also used.

Other carbohydrate standards were used to validate the ELSD parameters. β-galacturonate: apple pectin (Cat. #: 76282, Sigma, Alcobendas, Madrid, Spain). Alpha-galacto-oligosaccharides (α-GOS): raffinose (Cat. #: 217410; MW = 594.52 Da, Difco, Madrid, Spain), stachyose (Cat. #: S4001; MW = 666.58 Da, Sigma, Alcobendas, Madrid, Spain), verbascose (Cat. #: 120801; MW = 828.72 Da, Megazyme, Wicklow, Ireland). Disaccharides: cellobiose (Cat. #: 219458; MW = 342.3 Da, Merck, Darmstadt, Germany) and sucrose (Cat. #: 5737; MW = 342.3 Da, Merck, Darmstadt, Germany).

Food-grade commercial samples were also used for validation. Fructo-oligosaccharides (FOS): Raftilose® P95 and Raftiline® HP-GEL (Orafti, Barcelona, Spain), and inulin (Cat. #: I2255; Sigma, Alcobendas, Madrid, Spain).

All other reagents used were of chromatography grade. All solutions, dilutions and mobile phases were prepared with ultrapure water (Resistivity 18.2 M Ω cm at 25 °C; Milli-Q Integral 5 Water Purification System from Millipore, Merck KGaA, Darmstadt, Germany).

2.2. Instrument

The HPLC system was equipped with the following instruments: Kontron 360 autosampler, Agilent quaternary pump system 1200 series with online degasser, Jones chromatography thermostatic oven, Agilent HPLC control unit 1100 series and Agilent 380-ELSD Evaporative Light Scattering Detector. Data acquisition and analysis were performed with the OpenLAB CDS ChemStation from Agilent Technologies.

The analysis of carbohydrates was performed by high performance size exclusion (Gómez-Ordóñez et al., 2012) on a TSK gel G 5000 PW stainless steel column (300 mm × 7.5 mm) with a TSK-Gel PWH guard column (75 mm × 7.5 mm) from TosoHaas (Cat. #: TH-05764 and TH-06762, respectively; Teknokroma, Barcelona, Spain).

2.3. Optimization of ELSD parameters

To optimize the response of ELSD detection for carbohydrate analysis, including polysaccharides and oligosaccharides, a response surface methodology using Box–Behnken design was performed. Three independent variables were included: nebulizer temperature, evaporator temperature and gas (N_2) flow rate (additional Table 5). Other parameters of the ELSD were fixed: led intensity 100%, photomultiplier (PMT) gain 1.0, data rate 80 Hz and smoothing 3.0 s.

The dependent variable was the peak area in the chromatogram; noise signal was not considered because it did not change with experimental conditions. The RSM was carried out with the following carbohydrate standards: pullulan P-100 and P-50, dextran T-10 and maltodextrin. For a faster optimization of ELSD response, a direct injection of individual standards (100 μg L^{-1}), without the chromatography column (Lafosse & Herbreteau, 2002), was used with ultrapure water as the mobile phase.

A second order polynomial Eq. (1) was used to express the relation between the dependent variable Y and independent variables X_i, X_j. β_0 is the intercept, β_i is the linear coefficient, β_{ii}, the quadratic coefficient, β_{ij}, the interaction coefficient and ε, the residual. The software Design Expert version 9 trial was used to design the RSM experiments (Box–Behnken design) (additional Table 5) and to perform the response surface regression and the analysis of variance ($P < 0.05$).

$$Y = \beta_0 + \Sigma\beta_i X_i + \Sigma\beta_{ii} X_i^2 + \Sigma\beta_{ij} X_i X_j + \varepsilon \quad (1)$$

The mobile phase flow rate was used at 0.8 mL min^{-1} and the temperature of the chromatography oven was maintained at 40 °C as previously reported (Gómez-Ordóñez et al., 2012). Aqueous solutions of standards and samples were filtered through 0.45 μm filters (cellulose acetate filters, 25 mm diameter, Análisis Vínicos, Tomelloso, Toledo, Spain) and injected (50 μL) into the HPLC.

2.4. Validation of ELSD parameters

Once the ELSD parameters were experimentally optimized by RSM and direct injection without column, carbohydrates with high-, medium- and low-molecular weight (Blue dextran T-2000, MW = 2000 kDa; dextran T-500, MW = 500 kDa and maltodextrin, MW = 3.4 kDa) at 1 mg L^{-1} were injected onto an TSK-Gel G 5000 PW size-exclusion column and eluted with the mobile phase at flow rate of 0.8 mL min^{-1}.

In order to improve the peak shape of carbohydrates, depending on their high-, medium- or low-molecular weight, ultrapure water, 0.05 mol L^{-1} acetic acid, ammonium acetate at 0.05 mol L^{-1} and 0.01 mol L^{-1} were run isocratically and evaluated as mobile phases.

2.4.1. Linearity, sensitivity, and detection and quantitation limits of carbohydrate standards

Different carbohydrate standards (high-, medium- or low-molecular weight) were used for calibration: dextran standards (T-500, T-70 and T-10), pullulan P-100, maltodextrin (16.5–19.5 glucose equivalents) and glucose. Each standard in triplicate at various concentrations (250–1000 mg L^{-1}), with a mobile phase of 0.01 mol L^{-1} ammonium acetate was separately injected into the chromatographic column under the optimal conditions selected above for the ELSD. Regression standard curves were obtained for the logarithm of the molecular weight versus the logarithm of detection area from the ELSD (in mV*s). The limits of detection (LOD) and quantitation (LOQ) were calculated using the approach based on signal-to-noise ratio from sample solutions with known low concentrations (250 mg L^{-1}) of standard and blank solutions (Yang, Hu, & Zhao, 2011). LOD was calculated as the concentration corresponding to three times the peak height of the baseline noise (signal-to-noise ratio equals 3); whereas LOQ was set as 10 times