An Acetate–Hydroxide Gradient for the Quantitation of the Neutral Sugar and Uronic Acid Profile of Pectins by HPAEC-PAD without Postcolumn pH Adjustment

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Supporting information available free of charge via the Internet at http://pubs.acs.org.

SUPPORTING INFORMATION

An HPAEC-PAD method was developed and validated to quantitate seven neutral sugars and two uronic acids of hydrolyzed pectic polysaccharides without the need for postcolumn pH adjustment. The results of the validation experiments are discussed in the main document. There, the gradient profile used and the limits of quantification are presented in the Tables 1 and 2, respectively, while representative standard chromatograms are shown in Figure 1. In addition, the present supporting information includes three supplemental figures concerning the column performance under different conditions (Figure S1), the sensitivity of the method (Figure S2), and its linearity and range (Figure S3), respectively.

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Figure S1. HPAEC-PAD chromatograms for 10 µL of a standard solution (GalUA, 85.2 µmol L^{-1}; GlcUA, neutral sugars, 14.2–18.9 µmol L^{-1}): Separation, using either (A) a column temperature of 28 °C and the gradient profile of Table 1 with 200 mM NaOH for initial regeneration and 3.4 mM acetate plus 2 mM NaOH for equilibration or (B, C) only 16 °C and a modified gradient profile that involved 500 mM NaOH for strongly alkaline regeneration (steps *a*, *g*: 100% eluent *C*) and an elevated concentration of 10.2 mM acetate plus 6 mM NaOH (step *b*: 94% eluent *A*, 6% eluent *B*) for equilibration, which was additionally extended by delaying step *c* by 0.5 min (to +0.5 min at the expense of a shortened isocratic phase 3). For detection, either the brand-new (C) or the manually polished (A, B) nondisposable gold electrode was used. For peak assignment, cf. Figure 1 of the main document.

Figure S2. Sensitivity of the HPAEC-PAD method variant M_{Dx1} (injection volume: 25 µL) regarding galactose (Gal), glucuronic acid (GlcUA), and rhamnose (Rha): Regression lines ($y = s_{be}\, x + y_0$) at the bottom ends of the ranges for the estimation of the limits (Table 2 of the main document) according to DIN 32645 [24] from the means of ten measurements ($m = 1$) for each of seven standard solutions ($n = 7$) covering concentrations of 0.06–3.42 µmol L^{-1}. Error bars represent the standard deviations. R^2, coefficient of determination; $x = c$, concentration; y = Area, peak area; s_{be}, slope; y_0, ordinate intercept.

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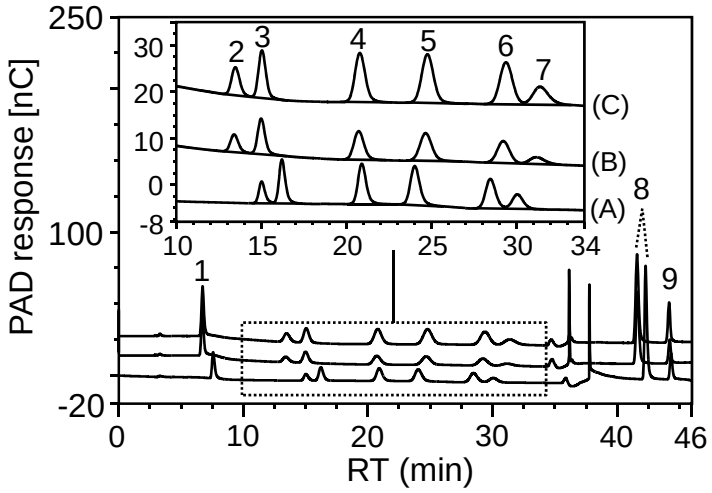
Figure S3. Linearity and range of the HPAEC-PAD method variant M_{Dx1}: Calibration lines ($y = s\, x$) through the origin and the respective means of 10 consecutive 6-point calibration series for (**A**) fucose (Fuc), rhamnose (Rha), and galactose (Gal); (**B**) glucose (Glc), xylose (Xyl), and mannose (Man); (**C**) glucuronic acid (GlcUA) and arabinose (Ara); and (**D**) galacturonic acid (GalUA). The concentrations of the standard solutions (white symbols) covered a range of 1.7–128 µmol L^{-1} for GalUA and 0.28–30.3 µmol L^{-1} for the other analytes. Black symbols indicate the limit of quantification (LOQ, Table 2) for each analyte. Error bars represent the standard deviations. R^2, coefficient of determination; $x = c$, concentration; y = Area, peak area; s, slope.



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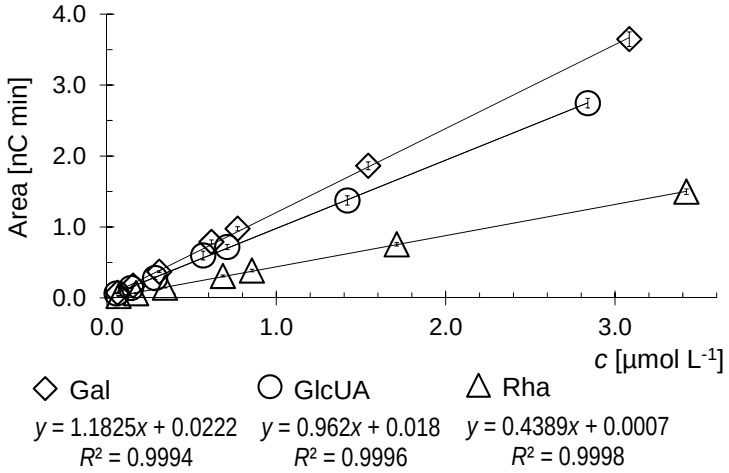

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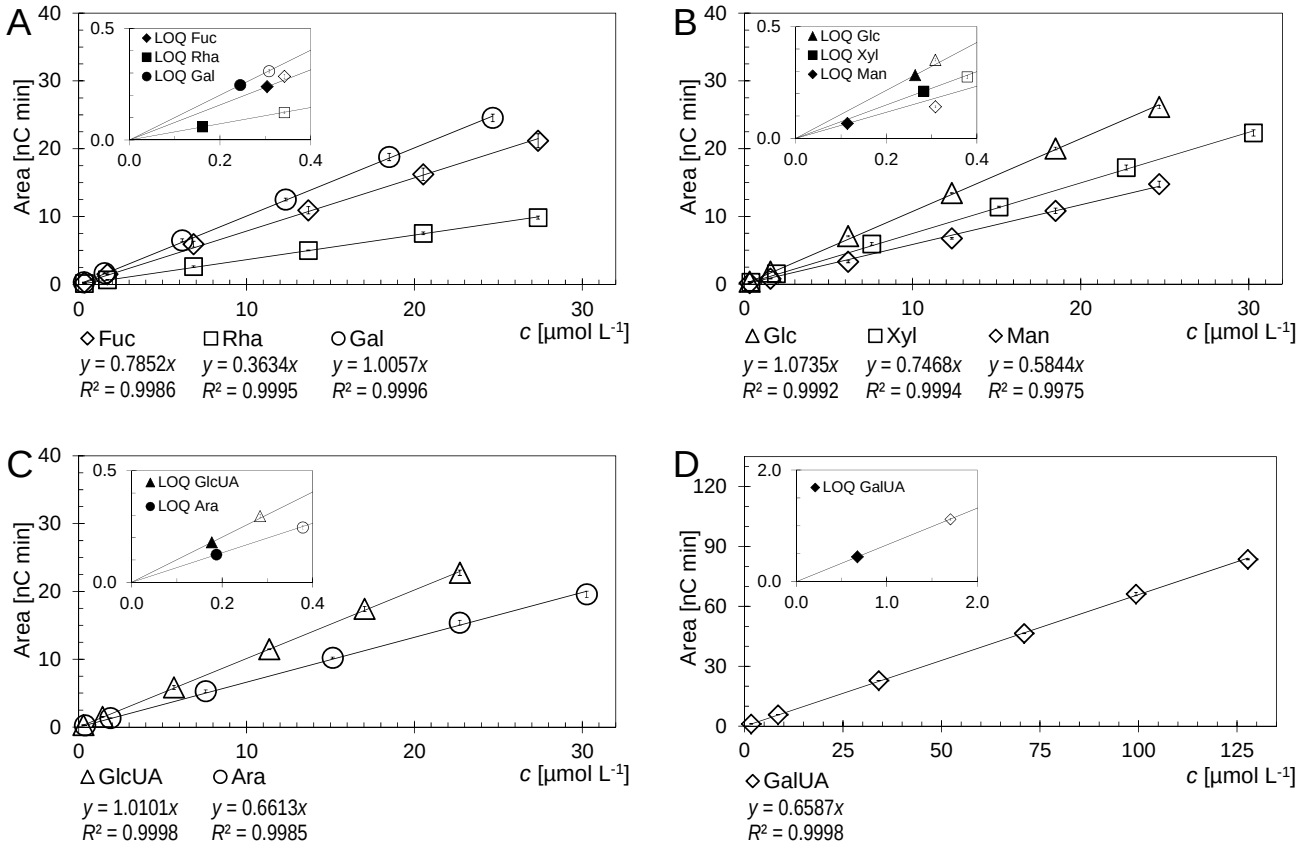

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