SUPPORTING INFORMATION:

Characterization of Complex Polyether Polyols Using Comprehensive Two-dimensional Liquid Chromatography Hyphenated to High-Resolution Mass Spectrometry

Gino Groeneveld¹*, Melissa N. Dunkle², Marian Rinken³, Andrea F. G. Gargano¹, Ayako de Niet¹, Matthias Pursch³, Edwin P.C. Mes², Peter J. Schoenmakers¹

¹University of Amsterdam, Van ’t Hoff Institute for Molecular Sciences, Science Park 904, 1098 XH Amsterdam, The Netherlands
²Dow Benelux B.V., Analytical Science, P.O. Box 48, 4530 AA Terneuzen, The Netherlands
³Dow Deutschland Anlagengesellschaft mbH, Analytical Sciences, P.O. Box 1120, 21677 Stade, Germany
⁴Vrije Universiteit Amsterdam, Amsterdam Institute for Molecules, Medicines and Systems, de Boelelaan 1083, 1081HV Amsterdam, The Netherlands

(*) Corresponding author
Science Park 904, 1098 XH Amsterdam, The Netherlands
E-mail: G.Groeneveld@uva.nl
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Figure S1: Increased resolution was observed for the separation of Gly-EO/PO when the column was cooled to 10°C compared to 23°C. Especially for the higher MW part of the sample, resolution was improved under sub-ambient conditions. Conditions: Kinetex HILIC (150 × 2.1 mm, 2.6 µm particles). MPA = 10 mM ammonium formate pH 3.2, MPB = 100% ACN. F = 0.4 mL/min; T = 10 or 23°C. Gradient: 3 min hold at 97% B followed by linear gradient to 70% B in 30 min, hold for 2 min and followed by re-equilibration at 97% B for 5 min. ELSD detection. 1µL, 10 mg/mL injection of Gly-EO/PO in 100% ACN.
**Figure S2:** (a) RP separation of Gly-EO in ACN with varying initial percentage of organic modifier (starting point of the gradient, % $B_{\text{init}}$) and relative small injection volumes ($V_{\text{inj}} = 2$ µL). As can be seen, higher % $B_{\text{init}}$ decreases overall retention time of the retained peaks while increasing the intensity of the breakthrough peak. At 50% $B_{\text{init}}$, a small part of the sample is slightly retained, eluting close to the breakthrough peak. (b) When decreasing the ACN fraction present in the injection solvent (which is the case when performing the 1D HILIC separation), the retained peak becomes more abundant and is slightly better resolved from the breakthrough peak.

Chromatographic conditions (a): column = Zorbax Eclipse RRHD C18 (50 × 2.1, 1.8-µm fully porous particles), MPA = deionized H$_2$O, MPB = MeOH, column temperature = 25°C, flow rate = 0.5 mL/min, injection volume = 2 µL, sample concentration = 5 mg/mL, gradients applied with varying % $B_{\text{init}}$ hold for 0.5 min, followed by a linear gradient of 32% B / min to 100% B, hold for 1 min followed by re-equilibration at specified % $B_{\text{init}}$.

Chromatographic conditions (b): column = Zorbax Eclipse RRHD C18 (50 × 2.1, 1.8 µm fully porous particles), MPA = deionized H$_2$O, MPB = MeOH, column temperature = 25°C, flow rate = 0.5 mL/min, injection volume = 2 µL, sample concentration = 5 mg/mL in varying % ACN, 50% $B_{\text{init}}$ hold for 0.5 min, followed by a linear gradient of 32% B / min to 100% B, hold for 1 minute followed by re-equilibration at 50% $B_{\text{init}}$. 

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Figure S3: Selected-ion chromatogram LC×LC-HRMS of COE-20 in negative-ionization mode, showing additional species compared to positive mode, due to reduced ionization efficiency under the latter conditions.
Figure S4: HILIC×RPLC-(+)HRMS separation of castor oil reacted with 40 mole equivalents of ethylene oxide (COE-40). In the first dimension, the degree of ethoxylation was resolved under HILIC conditions, while the 2D RPLC separated the various species according to hydrophobicity. With respect to the separation of COE-20, higher-molecular-weight solutes were separated without losing separation efficiency.
Figure S5: MS/MS spectra of additional isomer series showing distinct fragmentation patterns. The additional compounds subjected to LC-MS/MS measurements were (a) [Gly-Ric-20EO + 2NH₄]²⁺, (b) main and isomer peak of [Gly-RicRic-20EO + 2NH₄]²⁺, (c) [Gly-Lin-20EO + 2NH₄]²⁺, (d) isomer and main peak of [Gly-RicLin-20EO + 2NH₄]²⁺, and (e) isomer II and main peak of [Gly-RicRicLin-20EO + 2NH₄]²⁺ (isomer I was too low in abundance to obtain reliable MS/MS results). Neutral losses and identified fragment ions are shown in the corresponding spectra.
Figure S5: continued.