

Descriptions of greenness assessments utilized:

EHS Scores Using the GSK Guide: EHS scores are metrics utilized to quantify greenness and are often incorporated into solvent selection guides. These scores analyze various physicochemical properties associated with environmental, health, and safety hazards. Table S1 lists the properties and scoring criteria used in the GSK solvent selection guide.¹ Additional information on each property can be found in the Supplemental Information.

Each property is assigned a score, as described by Alder et al.,¹ from one to four, where larger values indicate greener solvents. Then, the geometric mean is taken of all the properties in a sub-category. Again, the geometric mean of each sub-category assigned to the Environmental, Health, and Safety categories is taken. Finally, the geometric mean of all three categories is taken to calculate an overall EHS Score. After the analysis of many solvents, scores were converted to a one to ten scale, however, that step is omitted here because only the most-commonly used solvents are considered herein, thus limiting our data set.

HPLC-EAT: HPLC-Environmental Assessment Tool (EAT)² is one of the first greenness metrics developed specifically for chromatographic applications. This assessment utilizes EHS scores, as well as the mass of each solvent consumed in the separation. Typically, the EHS scores used in this assessment vary between zero and one, where lower scores indicate a greener solvent. Equation S1 describes the calculation to determine HPLC-EAT Scores, where m is the mass of the solvent consumed and EHS is the EHS Score of the solvent. This is summed for all solvents in the mobile phase. This score only reflects hazards of the solvents used.

$$HPLC - EAT = \sum m_1 EHS_1 \quad (S1)$$

AMGS Calculator: As previously described, the ACS GCI-PR recently developed the Analytical Method Greenness Score (AMGS) calculator.³ This calculator consists of three categories that are summed to achieve a final greenness score. The Solvent EHS category considers the EHS score, and mass of each solvent consumed in the separation process. The use of the geometric mean to calculate EHS scores assists in ensuring that each category is weighted the same in the overall EHS score. The Solvent Energy category accounts for the CED and mass of each solvent consumed. CED values represent the energy needed to produce and dispose of a solvent. Finally, the Instrument Energy category accounts for the energy use of the instrument by considering the instrument type, the separation time, and the total number of injections in a full analysis. This assessment describes a more complete picture of the greenness of chromatographic techniques compared to the HPLC-EAT score.

References:

- 1 C. M. Alder, J. D. Hayler, R. K. Henderson, A. M. Redman, L. Shukla, L. E. Shuster and H. F. Sneddon, *Green Chem.*, 2016, **18**, 3879.
- 2 Y. Gaber, U. Törnvall, M. A. Kumar, M. A. Amin and R. Hatti-Kaul, *Green Chem.*, 2011, **13**, 2021–2025.
- 3 M. B. Hicks, W. Farrell, C. Aurigemma, L. Lehmann, L. Weisel, K. Nadeau, H. Lee, C. Moraff, M. Wong, Y. Huang and P. Ferguson, *Green Chem.*, 2019, **21**, 1816–1826.

Additional information regarding properties used to calculate EHS scores using the GSK solvent selection guide:

Acute toxicity, chronic toxicity, and biodegradation are used to assess hazardous conditions when solvents are present in natural aqueous environments. Acute toxicity is quantified using LC_{50} values for various aquatic species, which indicates the concentration of a chemical in the water that yields a mortality rate of 50%. Higher concentrations indicate a greener solvent, as a larger amount of the solvent would be needed to observe the required mortality. Chronic toxicity is measured with the partition coefficient ($\log K_{ow}$) of the solvent. Low $\log K_{ow}$ values indicate that the solvent will not persist in an aqueous environment which may lead to long term effects. Solvents that are biodegradable do not linger in the environment and are therefore green options.

Environmental hazards associated the presence of solvents in air can be determined by considering photochemical ozone creation potential (POCP) and solvent odor. POCP indicates the ability of volatile organic compounds (VOCs) to produce ozone via photochemical reactions in the atmosphere. Low PCOP values indicate minimal ozone production and greener solvents. The solvent odor category consists of a ratio of the vapor pressure and odor threshold of a solvent. Vapor pressure indicates how readily the solvent enters the gas state, which is easily transported in the atmosphere. Higher vapor pressures indicate the gas state is easily accessible, therefore lower vapor pressures indicate greener solvents. The odor threshold is the minimal concentration that can be detected by smell. The ratio of vapor pressure to odor threshold should be a low value to indicate a green solvent.

The health category can be quantified typically with information found on Safety Data Sheets, as well as exposure potential, which accounts for vapor pressure and occupational exposure limits (OEL). Solvent assigned specific GHS statements yield lower scores, thus having no relevant statements would provide a green solvent. Exposure potential scores consider the ratio between saturation concentration and occupational exposure limits. Saturation concentration is the vapor convert to parts per million (ppm). Higher exposure limits indicate that workers can be exposed to a large amount of solvent without significant health risks. Lower saturation concentration/OEL ratios indicate greener solvents.

In the safety category, both flammability and reactivity hazards are considered. To quantify flammability hazards, boiling point, flash point, and vapor pressure are considered. High boiling points indicate that less solvent will be in the gas phase, thus yielding lower flammability risk and a greener solvent. Flash point is the temperature at which the solvent gives off sufficient vapor to ignite. Solvent with high flash points have reduced flammability risk and are greener. Similarly, low vapor pressures suggest that less solvent will be in the gas phase, thus indicating a greener solvent due to the low flammability risk. Finally, the reactivity is quantified by considering the potential for peroxide formation and self-reaction, National Fire Protection Association (NFPA) hazard rating, acidity/basicity, and any additional hazards. Peroxide formation and self-reactions indicate greater chances for unregulated reactivity leading to unsafe conditions for storage and use. The NFPA hazard rating considers the stability of the solvent when exposed to high pressures and temperatures or reactions in water. The level of acidity and basicity also correspond to additional reactivity concerns. All of these parameters should be minimized to create a greener solvent.

Table S1 Summary of physiochemical properties used to calculate EHS scores via the GSK solvent selection guide¹

Category	Sub-category	Physiochemical Property	Scoring Criteria
Environment	Environment: Aqueous	Acute toxicity	Score = $\log(\text{worst case } LC_{50}) + 1$ Limits: 1 – 1000 mg/L
		Partition coefficient	Score = $-2 \times \log K_{ow} + 9$ Limits: $\log K_{ow} 2.5 - 4$
		Biodegradation	Score 4: Readily biodegradable or Biochemical Oxygen Demand (BOD) > 50 Score 3: BOD = 20-30 or stated to be inherently biodegradable Score 2: Slow to very slow rate of biodegradation Score 1: Data suggests a serious biodegradation issue
	Environment: Air	Photochemical ozone creation potential (POCP)	Score 4: POCP ≤ 20 Score 3: 20 > POCP ≤ 40 Score 2: 40 > POCP ≤ 60 Score 1: POCP > 60
		Odor threshold & Vapor Pressure	Score = $-0.44 \times \log(P_{vap}/OT) + 2.9$
Health	Health Hazard	GHS hazard phrases	Multiple GHS Statements for each score
		Occupational exposure limits	Only used if GHS Statements are not available
	Exposure Potential	Vapor hazard ratio (VHR)	Score = $-0.62 \times \log(VHR) + 4.56$
Safety	Flammability & Explosivity	Boiling point	Score = $0.043 \times BP - 0.71$ Limits: 40 – 110 °C
		Vapor pressure	Score = $-0.94 \times \log(VP) + 3.36$ Limits: 0.2 – 400 mm Hg
		Flash point	Score 4: FP > 50 °C Score 3: 20 °C ≤ FP ≤ 50 °C Score 2: 0 °C ≤ FP < 20 °C Score 1: FP < 0 °C
	Reactivity	NFPA reactivity rating	Score 4: NFPA Reactivity = 0 Score 3: NFPA Reactivity = 1-2 Score 2: NFPA Reactivity = 3 Score 1: NFPA Reactivity = 4
		Peroxide formation tendency	Score 4: No evidence of peroxide formation Score 3: Peroxide formation known under certain conditions Score 2: No data available but structure suggests the possibility of peroxide formation Score 1: Peroxide formation occurs readily
		Self-reactive hazard assessment	Score 4: None known Score 3: Some self-reaction known Score 2: Strong tendency for self-reaction Score 1: Very strong tendency for self-reaction
		Acidity/Basicity	Score 4: No acidity/basicity Score 3: Mild acid/base Score 2: Moderate acid/base Score 1: Strong acid/base
		Special hazards	Score 4: No special hazards Score 2: Risk of explosion on industrial process scale Score 1: Significant issues are known

Table S2: Summary of data collected to calculate EHS scores using the GSK solvent selection guide

Category	Sub-category	Physicochemical Properties	Calculations and Notes	Solvents						
				MeOH	EtOH	IPA	ACN	Hexane	Water	CO ₂
Environmental	Environmental Air	POCP		21.3 ^d	22.5 ^d	20.3 ^d	<20 ^f	45.2 ^a	<20	Not NOx or VOC ^j
		Vapor Pressure (mm Hg)		96 ^a	44.63 ^a	35.25 ^a	73.98 ^a	132 ^a	23.765	42923.4
		Odor Threshold (ppm)		8.9 ^e	10 ^b	22 ⁱ	170 ^b	130 ^b	Very high	44800 ^k
	Environmental Aqueous	LC50 (mg/L)		15400 ^a	9268 ^b	9640 ^a	400 ^a	2.1 ^a	Very high	59.12 ^m
		Partition Coefficient		-0.77 ^a	-0.1 ^a	0.05 ^a	-0.34 ^a	~4 ^a	Low	0.83 ^c
		Biodegradation		99% ^a	“bioaccumulation not expected” ^a	53% ^a	70% ^a	“Readily” ^b	Biodegradation product	Biodegradation product
Health	Exposure Potential	Saturation Concentration (ppm)	Vapor Pressure x (10 ⁶ /760)	126315.8	58723.7	46381.6	97342.1	173684.2	31269.7	56478157.9
		Occupational Exposure Limits (ppm)		200 ^a	1000 ^a	400 ^a	20 ^a	50 ^a	Large	5000 ^c
	Health Hazard	Health Hazard	Utilize either GHS Statements or OEL data	GHS Statement: H370 ^a	OEL Data: 1000 ppm ^a	OEL Data: 400 ppm ^a	OEL Data: 20 ppm ^a	OEL Data: 50 ppm ^a	OEL Data: >50 ppm	OEL Data: 5000 ppm ^c
Safety	Flammability & Explosivity	Boiling Point (°C)		64.7 ^a	78.3 ^a	82 ^a	81 ^a	69 ^a	100 ^a	-78.46 ^c
		Flash Point (°C)		9.7 ^a	14 ^a	12 ^a	6 ^a	-22 ^a	No ignition	No ignition ^c
		Vapor Pressure (mm Hg)		96 ^a	44.63 ^a	35.25 ^a	73.98 ^a	132 ^a	23.765	42923.4 ^c
	Reactivity	Peroxide Formation		None	None	Can react with air ^a	None	None	None	None
		Ability to Self-React		None	None	None	None	None	None	None
		NFPA-Reactivity Score		0 ^b	0 ^b	0 ^b	0 ^b	0 ^b	0	0 ^c
		Acidity/Basicity		None	None	None	None	None	None	None
Special Hazards		None		None	None	None	None	None		

^a Safety Data Sheet from Sigma Aldrich^b Safety Data Sheet from Fisher Scientific^c Safety Data Sheet from Airgas^d Y. Andersson-Sköld, P., et al. *J. Air Waste Manage. Assoc.*, 1992, **42**, 1152–1158^e Hellmen, T.M., et al. *J. Air Pollut. Control. Assoc.*, 1974, **24**, 979-982^f <https://www.epa.gov/toxics-release-inventory-tri-program/acetone-nitrile-petition> (Accessed 3/15/21)^g <https://www.epa.gov/sites/production/files/2016-09/documents/hexane.pdf> (Accessed 3/15/21)^h CRC Handbook and Chemistry and Physics, 65th Edition, at 25 °Cⁱ <https://www.nj.gov/health/eoh/rtkweb/documents/fs/1076.pdf> (Accessed 3/15/21)^j <https://www.epa.gov/ground-level-ozone-pollution/ground-level-ozone-basics#formation> (Accessed 3/15/21)^k Melzner J., et al., *Chem. Senses*, 2011, **36**, 435–441.^m Furtado P.S, et al. *Mar. Freshw. Behav. Physiol.*, 2016, **49**, 337–346.

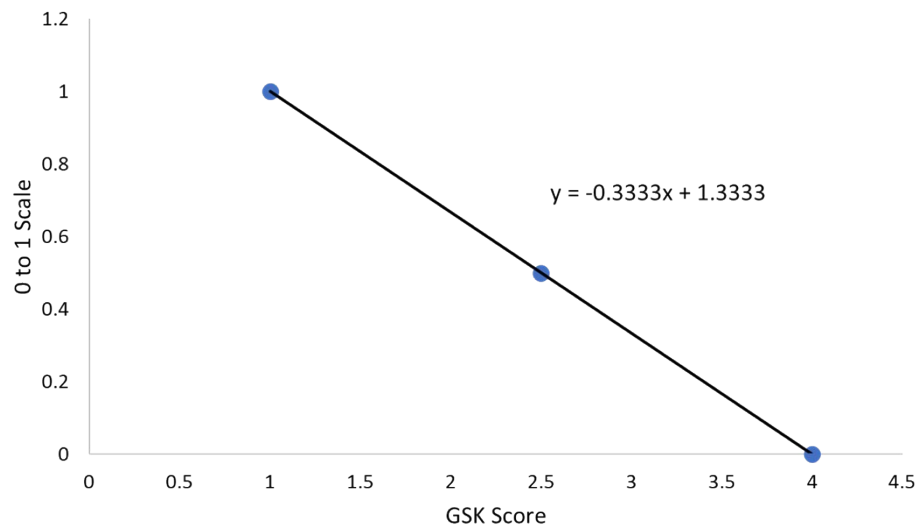


Figure S1: Linear fit utilized to convert EHS scores from the GSK guide, which are on a 1 to 4 scale, where four indicates a green solvent, to a 0 to 1 scale, where 0 indicates a green solvent.

Table S3: EHS Scores for commonly used solvents at ambient and elevated conditions

	1 bar, 25 °C	50 bar, 25 °C	100 bar, 25 °C	200 bar, 25 °C	300 bar, 25 °C	400 bar, 25 °C	50 bar, 30 °C	200 bar, 40 °C	200 bar, 50 °C	200 bar, 60 °C
MeOH	0.496	0.458	0.458	0.458	0.458	0.458	0.463	0.473	0.482	0.483
EtOH	0.355	0.334	0.334	0.334	0.334	0.334	0.339	0.348	0.358	0.363
IPA	0.428	0.407	0.407	0.407	0.407	0.407	0.411	0.420	0.428	0.436
ACN	0.512	0.487	0.487	0.487	0.487	0.487	0.491	0.500	0.508	0.517
Hexane	1.009	0.967	0.967	0.967	0.967	0.967	0.973	0.984	0.992	0.992
Water	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.003
CO ₂	0.036	0.036	0.036	0.036	0.036	0.036	0.036	0.036	0.036	0.036

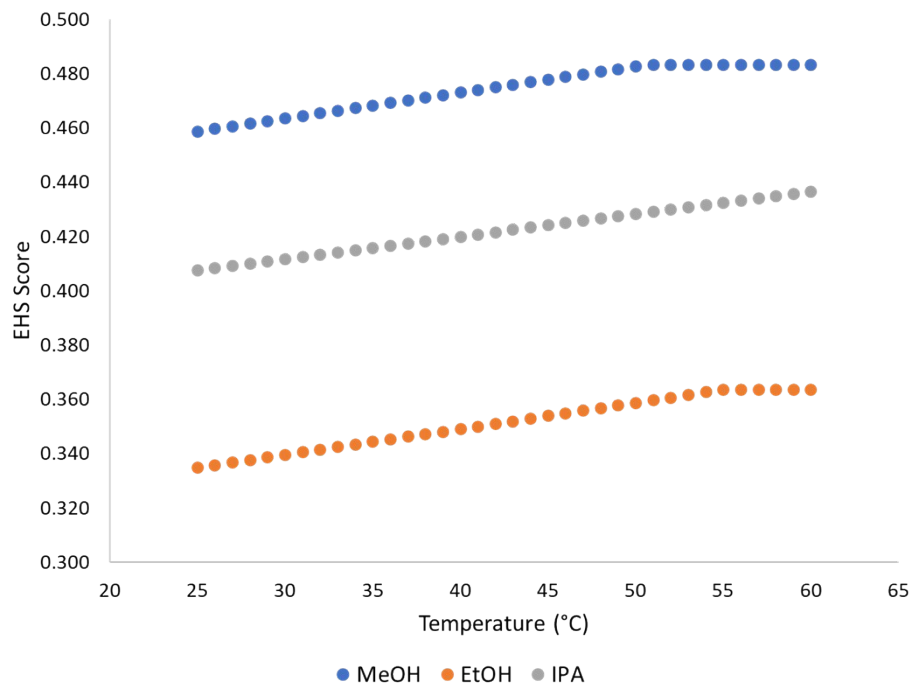


Figure S2: Effect of temperature on EHS Scores of common alcohols. These scores are valid for all pressures greater than 50 bar. The linear relationship ends at 51 °C for methanol and 55 °C for ethanol.

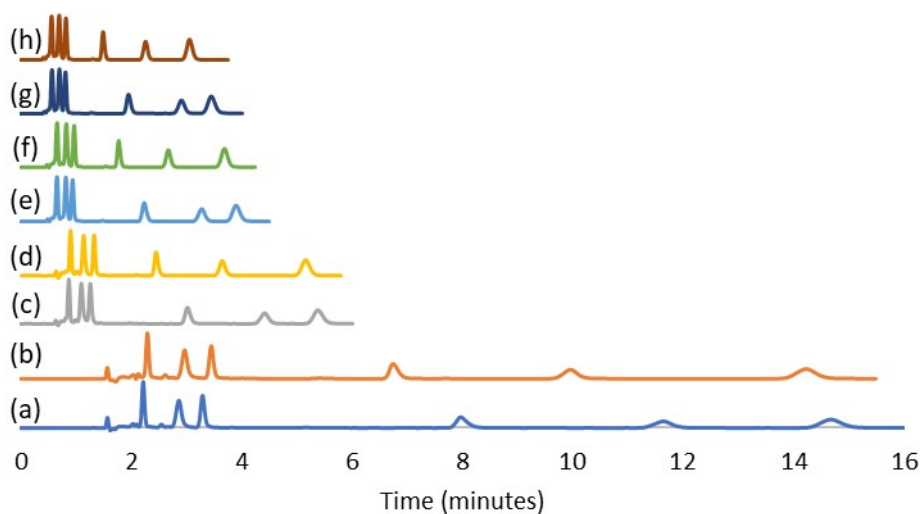


Figure S3: SFC separations with 22% MeOH+0.1% TEA/78% CO₂ of the high polarity mixture at various pressures and two temperatures used to evaluate the accuracy of several density values. (a) 150 bar, 1 mL/min, 25 °C, (b) 150 bar, 1 mL/min, 40 °C, (c) 200 bar, 2.5 mL/min, 25 °C, (d) 200 bar, 2.5 mL/min, 40 °C, (e) 250 bar, 3.25 mL/min, 25 °C, (f) 250 bar, 3.25 mL/min, 40 °C, (g) 300 bar, 3.75 mL/min, 25 °C, (h) 300 bar, 3.75 mL/min, 40 °C. The BPR for all separations was set to 130 bar and 60 °C. Retention order is: progesterone, flurbiprofen, prednisolone, diltiazem, amitriptyline, thioridazine.

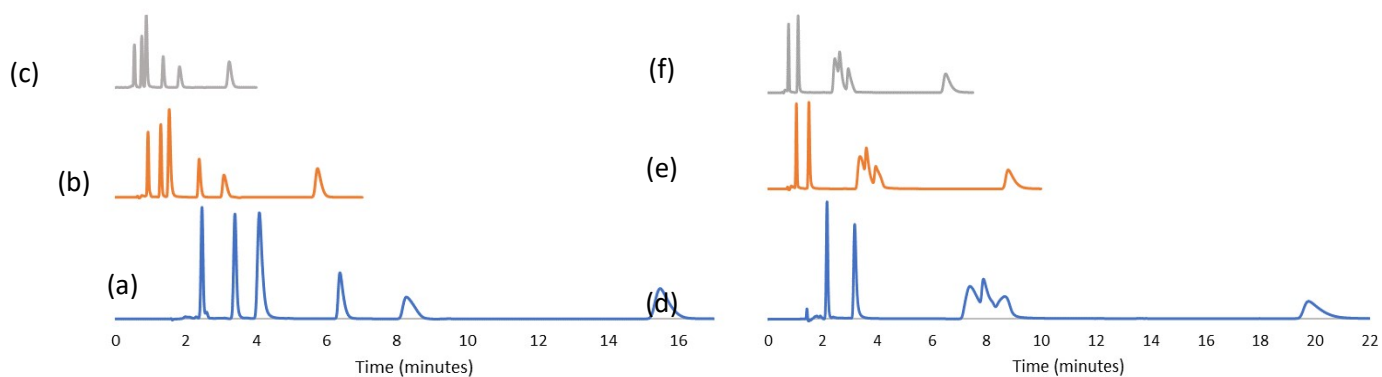


Figure S4: (a-c) SFC separations with 27% EtOH+0.1% TEA/73% CO₂ of the high polarity mixture at various pressures to evaluate the accuracy of several density values. (a) 150 bar, 1 mL/min, (b) 200 bar, 2.5 mL/min, (c) 300 bar, 4 mL/min. Temperature was constant at 50 °C. (c-f) SFC separations with 25% IPA+0.1% TEA/75% CO₂ to evaluate the accuracy of several density values (d) 150 bar, 1.25 mL/min, (e) 200 bar, 2.5 mL/min, (f) 250 bar, 3.3 mL/min. The temperature was constant at 40 °C. The BPR for all separations was set to 130 bar and 60 °C. Retention order is: progesterone, flurbiprofen, prednisolone, diltiazem, amitriptyline, thioridazine.

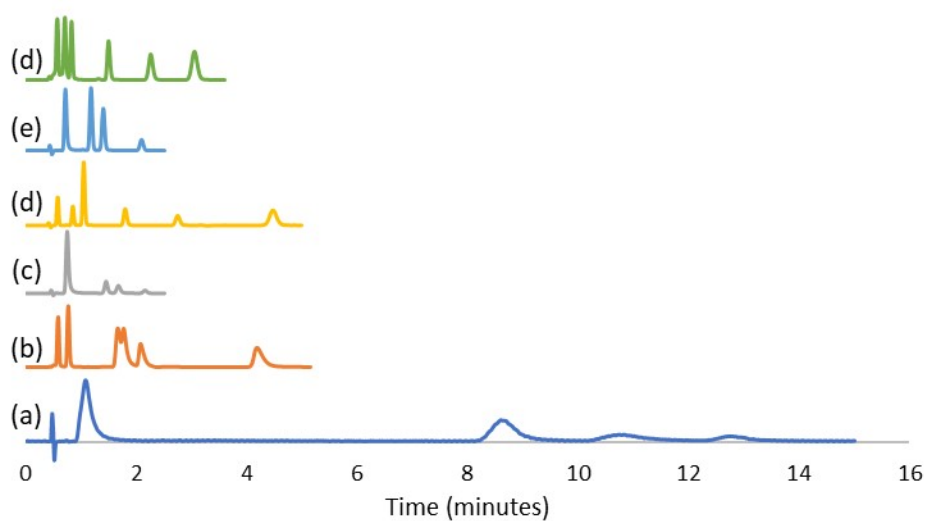


Figure S5: SFC separations of the high and low polarity mixtures utilizing various alcohols in the mobile phase. (a) low polarity mixture, 6% IPA, (b) high polarity mixture, 25% IPA, (c) low polarity mixture, 12% EtOH, (d) high polarity mixture, 27% EtOH, (e) low polarity mixture, 12% MeOH, (f) high polarity mixture, 22% MeOH. Mobile phase additives include 0.1% TEA and 0.1% AA for the high polarity and low polarity mixtures, respectively. The temperature of the column compartment is held at 40 °C for all separations. Flow rate for the high and low polarity mixtures is 3.75 mL/min and 4 mL/min, respectively, both of which correspond to a system pressure of 300 bar. The BPR is set to 130 bar and 60 °C. Retention order for the high polarity mixture is: progesterone, flurbiprofen, prednisolone, diltiazem, amitriptyline, thioridazine. The elution order for the low polarity mixture is: ketoprofen, hydrocortisone, prednisone, chlorthalidone.

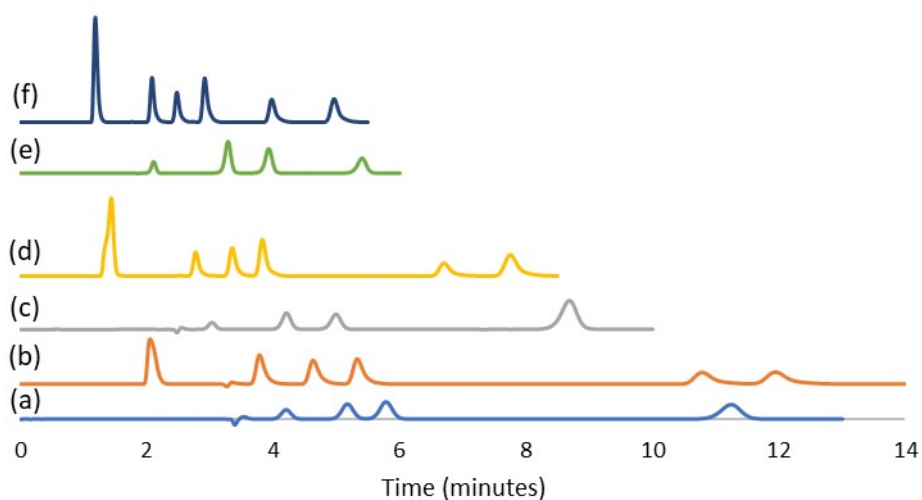


Figure S6: LC separations of the high and low polarity mixtures utilizing various organic solvents in the mobile phase. (a) low polarity mixture, 35% IPA, (b) high polarity mixture, 60% IPA, (c) low polarity mixture, 45% EtOH, (d) high polarity mixture, 75% EtOH, (e) low polarity mixture, 65% MeOH, (f) high polarity mixture, 90% MeOH. Mobile phase additives include 0.1% TEA and 0.1% AA for the high polarity and low polarity mixtures, respectively. The temperature of the column compartment is held at 40 °C for all separations. Flow rate for MeOH separation is 1 mL/min, IPA separations is 0.5 mL/min, and EtOH separations is 0.7 mL/min. Retention order for the high polarity mixture is: progesterone, flurbiprofen, prednisolone, diltiazem, amitriptyline, thioridazine. The elution order for the low polarity mixture is: ketoprofen, hydrocortisone, prednisone, chlorthalidone.

Figure S7. Impact of changes in EHS score on all solvents for both Solvent EHS category (blue) and overall AMGS (grey) for LC separations using both the high and low polarity mixtures. Solid bars indicate scores using the EHS scores from the AMGS calculator. Striped bar indicate changes resulting from changes in EHS scores.

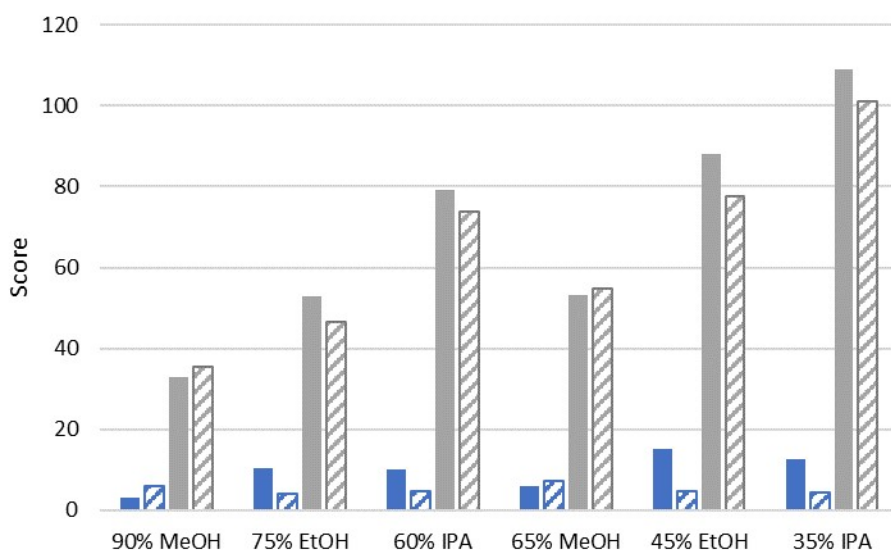


Table S4. Example calculation of composition of mobile phase released from leak. Conditions include operating at 150 bar and 30 °C with a mobile phase of 40% MeOH/60% CO₂. Areas highlighted yellow indicate values input by the user. Information about each calculation is described.

			Leak Time ⁱ (s)	Mass Mobile Phase Released ^j (kg)	Mass of Organic Released ^k (kg)	Mass of CO ₂ Released ^k (kg)	Volume of Organic Released ^m (mL)	Volume of CO ₂ Released ^m (mL)	Total Volume of Column Compartment ⁿ (mL)	% MeOH in Column Compartment ^p	% CO ₂ in Column Compartment ^p
ID of Tubing (cm)	0.07		1	0.02725	0.01037	0.01689	13.09076	19.63614	1432.72690	0.91	1.37
Area of Tubing ID (m ²) ^a	3.484*10 ⁻⁷		2	0.05451	0.02074	0.03377	26.18152	39.27228	1465.45380	1.79	2.68
Operating Pressure (bar)	150		3	0.08176	0.03110	0.05066	39.27228	58.90842	1498.18070	2.62	3.93
Operating Pressure (Pa) ^b	2.5*10 ⁷		4	0.10902	0.04147	0.06755	52.36304	78.54456	1530.90760	3.42	5.13
Operating Temperature (°C)	30		5	0.13627	0.05184	0.08444	65.45380	98.18070	1563.63450	4.19	6.28
Operating Temperature (K) ^c	313.15		6	0.16353	0.06221	0.10132	78.54456	117.81684	1596.36140	4.92	7.38
Specific Heat Ratio (CO ₂)	1.3		7	0.19078	0.07258	0.11821	91.63532	137.42598	1629.08830	5.62	8.44
Universal Gas Constant (R; J/kg*K)	188.9		8	0.21804	0.08294	0.13510	104.72608	157.08912	1661.81519	6.30	9.45
Volume of Air (cm ³) ^d	1400		9	0.24529	0.09331	0.15198	117.81684	176.72526	1694.54209	6.95	10.43
Volume of Organic (mL) ^e	0.40		10	0.27255	0.10368	0.16887	130.90760	196.36140	1727.26899	7.58	11.37
Volume of CO ₂ (mL) ^e	0.60		11	0.29980	0.11405	0.18576	143.99836	215.99754	1759.99589	8.18	12.27
Density of Organic (g/mL)	0.792		12	0.32706	0.12441	0.20264	157.08912	235.63367	1792.72279	8.76	13.14
Density of CO ₂ (g/mL)	0.860		13	0.35421	0.13478	0.21953	170.17988	255.26981	1825.44969	9.32	13.98
Mass Organic (g) ^f	0.17424		14	0.38157	0.14515	0.23642	183.27064	274.90595	1858.44969	9.86	14.79
Mass of Organic (kg)	0.00017424		15	0.40882	0.15552	0.25331	196.36140	294.54209	1890.90349	10.38	15.58
Mass of CO ₂ (g) ^f	0.6708		16	0.43608	0.16589	0.27019	209.45216	314.17823	1923.63039	10.89	16.33
Mass of CO ₂ (kg)	0.0006708		17	0.46333	0.17625	0.28708	222.54292	333.81437	1956.35729	11.38	17.06
Mass of Mobile Phase (kg) ^g	0.000845		18	0.49059	0.18662	0.30397	235.63367	353.45051	1989.08419	11.85	17.77
Mass Flow Rate (kg/s) ^h	0.044694		19	0.51784	0.19699	0.32085	248.72443	373.08665	2021.81109	12.30	18.45
Constants in Equation 1			20	0.54510	0.20736	0.33774	261.81519	392.72279	2054.53799	12.74	19.11
\sqrt{k}/R	0.08296										
$\frac{k-1}{1+\frac{k-1}{2}}$	1.15										
$\frac{-k+1}{2(k-1)}$	-0.06522										

^a Area=($\pi(d/2)^2$)/10000

^b 1 bar = 10⁵ Pa

^c Kelvin = °C + 273.15

^d Volume of air is determining by measuring the volume of the column compartment

^e Volume of mobile phase components is determined by the separation parameters

^f Volume (mL) * density (g/mL)

^g Sum the mass of all mobile phase components

^h Utilize Equation 1 (found in primary paper)

ⁱ User choose, increments of 1 second is recommended

^j Mass flow rate (kg/s) * leak time (s)

^k Mass of mobile phase released (kg) * (Mass of component (kg)/mass of mobile phase (kg))

^m Mass of component released * (density/1000)

ⁿ Volume of air + Volume of Organic Released + Volume of CO₂ Released

^p (Volume of Component Released/Total Volume in Column Compartment)*100%

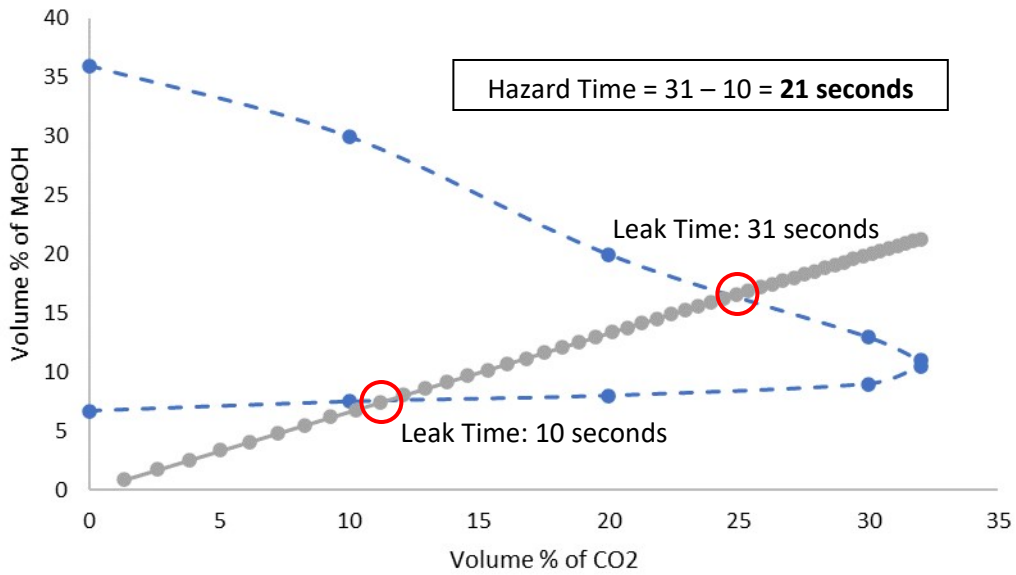


Figure S8: Example of methodology for calculating flammability hazard times. Flammability limits are of MeOH-CO₂-air mixtures. Data series in gray indicate the MeOH-CO₂-air compositions at various leaks time. Separations conditions include 150 bar and 30 °C with a 40/60 MeOH/CO₂ mobile phase.

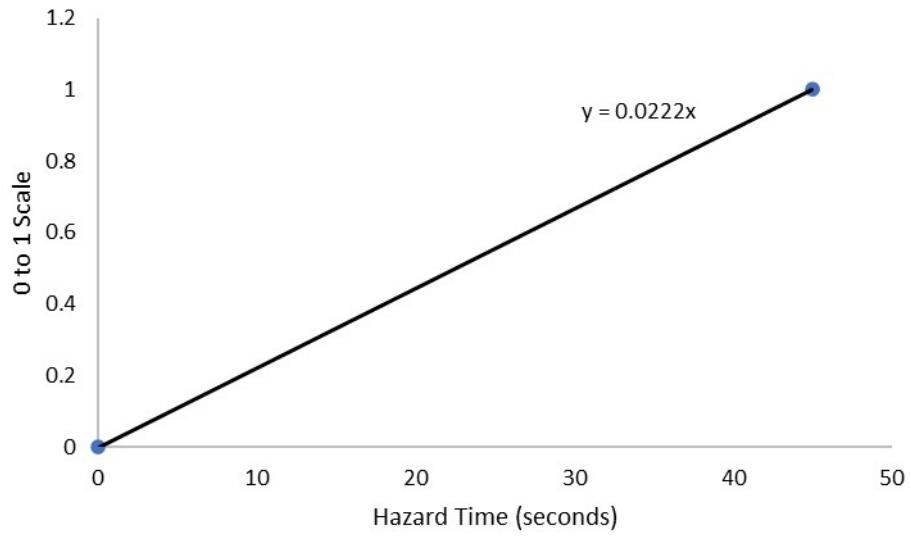


Figure S9. Linear fit utilized to convert hazard times to a 0 to 1 scale. Limits for hazard time range from 0 to 45 seconds.