Supplementary information

Long-chain alkanol–alkyl carboxylic acid-based low-viscosity hydrophobic deep eutectic solvents for one-pot extraction of anthraquinones from Rhei Radix et Rhizoma

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1. Supplementary experimental section

Polarity determination

First, 1.25 mM pyrene stock solution was prepared in anhydrous ethanol. A 50 μL of the pyrene stock solution was added in a 1.5 mL centrifuge tube, followed by vacuum-dried to remove ethanol. The residual pyrene was dissolved in different solvents (studied nine HDESs, other three reported HDESs, conventional hydrophilic DESs and organic solvents) under 10 min of ultrasound and 2 min of vortex. Then, the emission spectrum of the obtained solution was measured by fluorescence spectrophotometer (Hitachi F-460, Japan) at an excitation wavelength of 335 nm, and the fluorescence intensity ratio (I_1/I_3) of the first peak (I_1, 373.5±0.5 nm) and the third peak (I_3, 384.5±0.5 nm) was defined as the polarity of solvent.

Hydrophobicity and water-stability study of HDES

In order to initially explore the hydrophobicity of HDESs, 450 μL, 400 μL, 300 μL, 200 μL and 150 μL of HDES, as well as 150 μL, 200 μL, 300 μL, 400 μL, 450 μL of water (containing 0.1 mM rhodamine B as indicator) were added in a 1.5 mL centrifuge tube to obtain a HDES-water mixture at the volume ratio of 3:1, 2:1, 1:1, 1:2, 1:3, respectively. The mixture was vortexed for 10 min and centrifuged at 6000 rpm for 10 min, and then the appearance phenomenon was recorded. The hydrophobicity and water-stability of HDESs was further studied by evaluating mutual solubility of HDES and water. 1 mL of HDES and 1 mL of deionized water were mixed in a 5 mL centrifuge tube and then the mixture was vigorously stirred for 1 h in a magnetic heating agitator (DragonLab MS-H-Pro+, China). After centrifuging for 10 min at 6000 rpm, the top phase (HDES

phase) and bottom phase (water phase) were taken by a pipette and a syringe, respectively. The water content in the HDES phase was determined by Karl Fischer titration (Metrohm 890 Titrando, Switzerland). The HDES in the water phase was characterized by ^{1}H-NMR using d_6-DMSO as the deuterated reagent.

Quantification analysis of AQs by HPLC-DAD

The quantification of AQs were performed on a Dionex UltiMate 3000 HPLC system (Thermo Scientific) equipped with a quaternary pump, a diode array detector and a manual injection system. The data processing was carried out by Chromeleon 7.10 SR1 software. The separation was carried out using a Thermo Syncronis C_{18} column (250 mm × 4.6 mm, particle size 5 μm) with the column temperature at 40 ℃. The mobile phase was comprised of 0.1% H_3PO_4 water (A) and methanol (B), the gradient elution program was as follows: 0-10 min, 70%-75% B; 10-11 min 75%-85% B; 11-30 min 85%-95% B. The flow rate was set at 1.0 mL min^{-1}, the detection wavelength was at 254 nm, and the injection volume was 10 μL.

Experimental design strategy and statistical analysis for extraction optimization

The extraction conditions were first optimized by single-factor design experiment to determine the values of high and low levels of the extraction factors for Plackett-Burman design (PBD), which was further conducted to screen out the factors that significantly affect the extraction yield. The detailed PBD are shown in Table S5. Subsequently, response surface methodology (RSM) combined with Box-Behnken design (BBD) was adopted to optimize the factors chosen by PBD, namely, extraction temperature (A), liquid-solid ratio (B) and concentration of HCl (C) at three levels. The

established 17-run with five central points BBD project are shown in Table S6 with total AQs yield as the response.

Design Expert version 8.0.6 was used for the design and analysis of PBD and BBD. Besides, analysis of variance (ANOVA) and the lack-of-fit test were performed to evaluate the accuracy of the proposed model. All the experiments were carried out in random order and triplicate.

Pharmacopoeia method

A reference RRR sample was prepared according to the method listed in Volume 1 of Chinese pharmacopoeia [33]. Firstly, 0.15 g of RRR herb powder (65 mesh) was extracted with 25 mL of methanol by heat-refluxing for 60 min. The obtained solution was cooled to room temperature and then filtered. After that, 5 mL of the continuous filtrate was drawn, transferred to a flask and evaporated to dryness. 10 mL of 8% (v/v) HCl solution was then added and the mixed solution was sonicated for 2 min. Subsequently, 10 mL of chloroform was added and the mixture was heated to reflux for 1 h. After cooling to room temperature, the acid-hydrolyzed solution was transferred to a separation funnel and extracted three times with 10 mL of chloroform each time. The combined chloroform solution was evaporated to dryness under reduced pressure. And the residue was dissolved with methanol and transferred to a 10 mL volumetric flask. Then, methanol was added to the mark. After filtration, the reference RRR sample was obtained and directly used for detection of AQs by HPLC-DAD.

Solubility determination of AQs in HDESs

The solubility of AQs in nine HDESs, traditional DESs (ChCl–glycerin and ChCl–ethylene glycol), water and common organic solvents (methanol and ethanol) was determined by fully saturated

method with 1,8-dihydroxyanthraquinone (1,8-DHAQ) as a representative of AQs. Excess 1,8-DHAQ was added to a tested solvent, and the mixture was stirred (500 rpm) at 25°C for 2 h in order to supersaturate the solvent. After standing and centrifuging (8000 rpm for 10 min) for precipitation, the supernatant was diluted by mobile phase (95:5 v/v methanol-water) and the concentration of 1,8-DHAQ was analyzed by HPLC-DAD at wavelength of 254 nm. The standard curve and method validation results for 1,8-DHAQ analysis are shown in Table S8.

Method validation of C_{14} alcohol–UA DES based extraction method with HPLC-DAD for AQs quantification

The method validation was conducted by investigating analytical parameters including specificity, linear equation, linearity, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, and stability. The linear equation was constructed by plotting peak area of each AQ against eight concentrations of AQs methanol working solutions diluted from the stock solution. The LODs and LOQs were calculated at a signal to-noise (S/N) ratio of 3 and 10, respectively. The intra-day and inter-day precision were evaluated by analysis of 0.1 g RRR under the optimal extraction conditions. And the relative standard deviation (RSD) was credited as an indicator of precision. The accuracy of the method was assessed by studying the recovery of three concentration levels (low 50%, middle 100%, high 150%) of the spiked RRR samples. The relative recovery (RR) was calculated by the following equation:

$$RR\% = \frac{C_{found} - C_{real}}{C_{added}} \times 100\%$$

where C_{found}, C_{real}, C_{added} (mg/g) refer to the determined concentration of AQs in the spiked RRR, the concentration of AQs in the unspiked RRR, and the added concentration of AQs into the RRR sample, respectively. The stability of analytes was investigated by determining the content of analytes in the HDES phase, which was obtained after one-pot extraction, before and after 24 hours of standing.

2. Supplementary results and discussion

Method validation of C_{14} alcohol–UA DES based extraction method with HPLC-DAD for AQs quantification

The analytical performance of the proposed method for analysis of AQs in RRR was validated, the representative chromatograms are shown in Fig. S11 and the analytical parameters are summarized in Table S9 and Table S10. The DES and other extracted components do not interfere with the determination of five main AQs. The favorable linearity with correlation coefficients (R) higher than 0.9997 is achieved for five AQs within the chosen concentration ranges. The LODs and LOQs range from 0.008 to 0.018 μg/mL and 0.028 to 0.059 μg/mL, respectively. The RSDs for intra-day and inter-day precision are lower than 2.37% and 3.75%, respectively. And the recoveries are in the reasonable range of 86.9-102.6% with RSD less than 3.84%. The stability test exhibits that the analytes are highly stable in HDESs because no significant difference in analytes content exists before and after 24 h standing. The results above demonstrate that the method, C_{14} alcohol–UA HDES based extraction coupled with HPLC-DAD, is suitable for quantitative analysis of AQs in RRR sample.

Single factor optimization

Nine HDESs were chosen to screen extraction solvents of AQs from RRR sample. As shown in Fig. S9A, all the nine HDESs showed good extraction performance with total AQs yield more than 19.5 mg/g, but C_{14} alcohol–UA showed a relatively higher extraction yield. The reason is that C_{14} alcohol with longer alkyl chain can generate stronger hydrophobic interactions with AQs, and UA with an olefinic bond may have an extra π-π interaction with the benzene ring-containing analytes. Thus，C_{14} alcohol-UA was adopted as extraction solvent for further study.

The effect of different extraction methods, i.e. stirring (500 rpm, 60 °C, 60 min), water-bath (60 °C, 60 min), shaking (500 rpm, 60 °C, 60 min) and ultrasound (120 W, 60 °C, 60 min) on the extraction yield were further investigated. As seen in Fig. S9B, stirring was found as the most effective mean (21.09±0.17 mg/g). This is because continuous stirring can make the system be completely mixed, thus intensify the contact between the extraction solvent and target compounds. Although ultrasound can promote the contact between the extraction media and analytes to a certain extent, due to the low density and water-insolubility of HDES, ultrasound cannot achieve sufficient interaction between HDES, water and plant powder. Considering the best extraction effect as well as simple and convenient operation features without special extraction equipment, the stirring method was selected for subsequent research.

Extraction time and temperature play the important role in the extraction process of AQs. As seen in Fig. S9C, the yield of AQs rose up in the first 20 min and then remained unchanged, indicating 20 min of stirring can reach the equilibrium yield of this method. The effect of temperature on extraction of AQs was investigated with 20 min of stirring. As shown in Fig. S9D, total AQs yield increased significantly at first, then almost kept consistent with temperature more than 60 °C. This can be

139 explained by the fact that the increasing temperature can not only reduce the viscosity of C_{14} alcohol–
140 UA HDES, but also result in a quicker diffusion of AQs into HDES. Accordingly, 20 min of stirring
141 and 60 ^{o}C of extraction temperature were selected as the optimal conditions.

142 The rise in liquid-solid ratio can increase the concentration difference of analytes between
143 extraction solvent and raw materials, thereby enhancing the driving force of mass transfer. In this work,
144 six liquid-solid ratios were investigated and the results (Fig. S9E) suggested the AQs yield increased
145 apparently as liquid-solid ratio rose up from 5:1 to 10:1, and then reached equilibrium with further
146 increase. To avoid the waste of DES, 10:1 of liquid-solid ratio was selected as suitable ratio for
147 extracting AQs.

148 The influence of the concentration of HCl on the yield of total AQs (Fig. S9F) showed that 10%
149 (w/v) HCl was sufficient to provide the maximum yield, probably because the bound AQs (glycoside
150 form of free AQs) were totally hydrolyzed. Besides, the acidic environment facilitated AQs to maintain
151 their molecular form (pKa values in Table S1), thus reducing their distribution in the aqueous phase.
152 Hence, 10% (w/v) was deemed as the optimal HCl concentration.

3. Supplementary figures

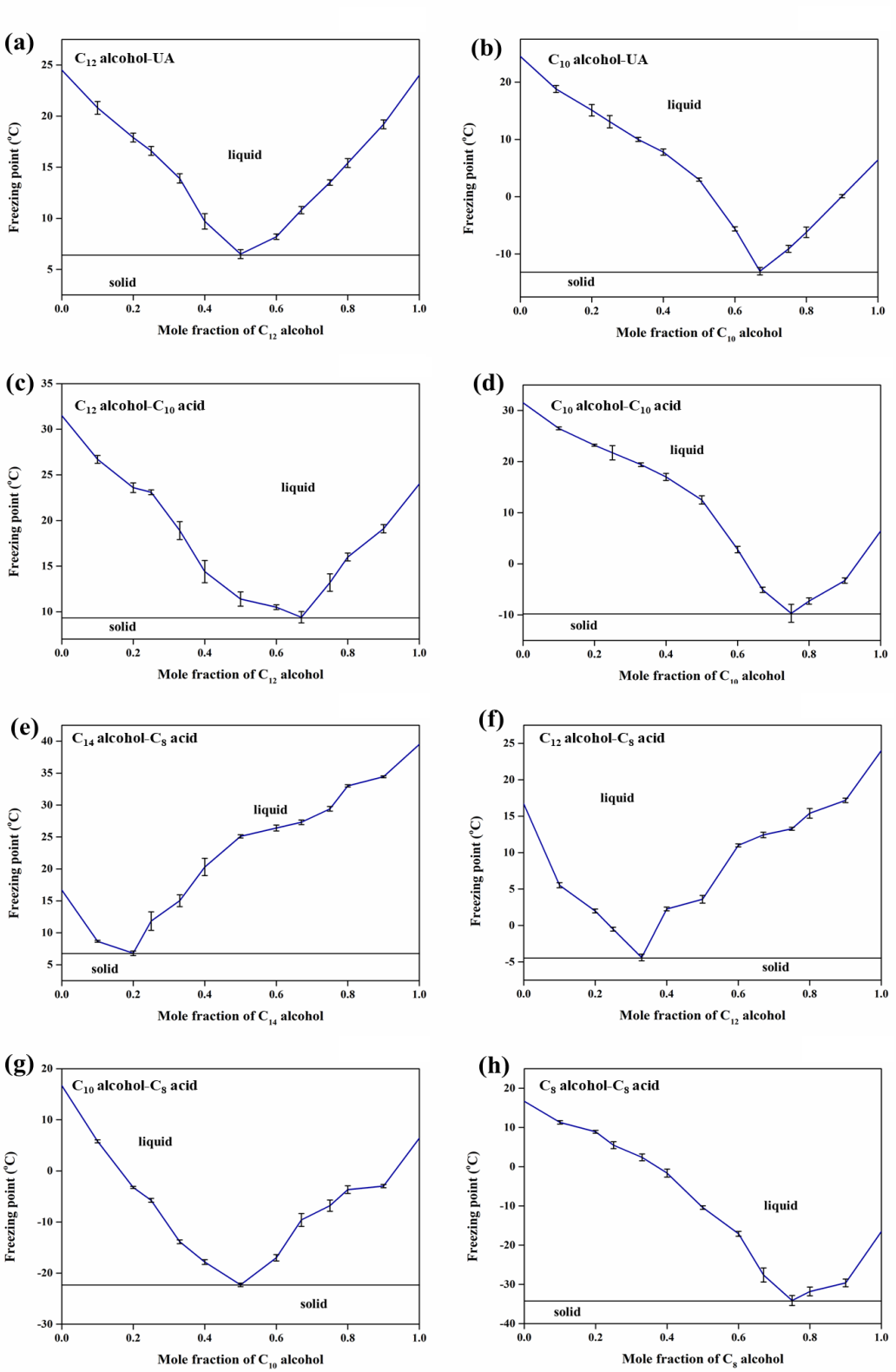


Fig. S1. Solid-liquid phase diagrams of C_{12} alcohol–UA (a), C_{10} alcohol–UA (b), C_{12} alcohol–C_{10} acid (c), C_{10} alcohol–C_{10} acid (d), C_{14} alcohol–C_8 acid (e), C_{12} alcohol–C_8 acid (f), C_{10} alcohol–C_8 acid (g) and C_8 alcohol–C_8 acid (f) HDESs, where above the blue curve is the liquid region, below the black line is the solid region. UA: 10-undecenoic acid.

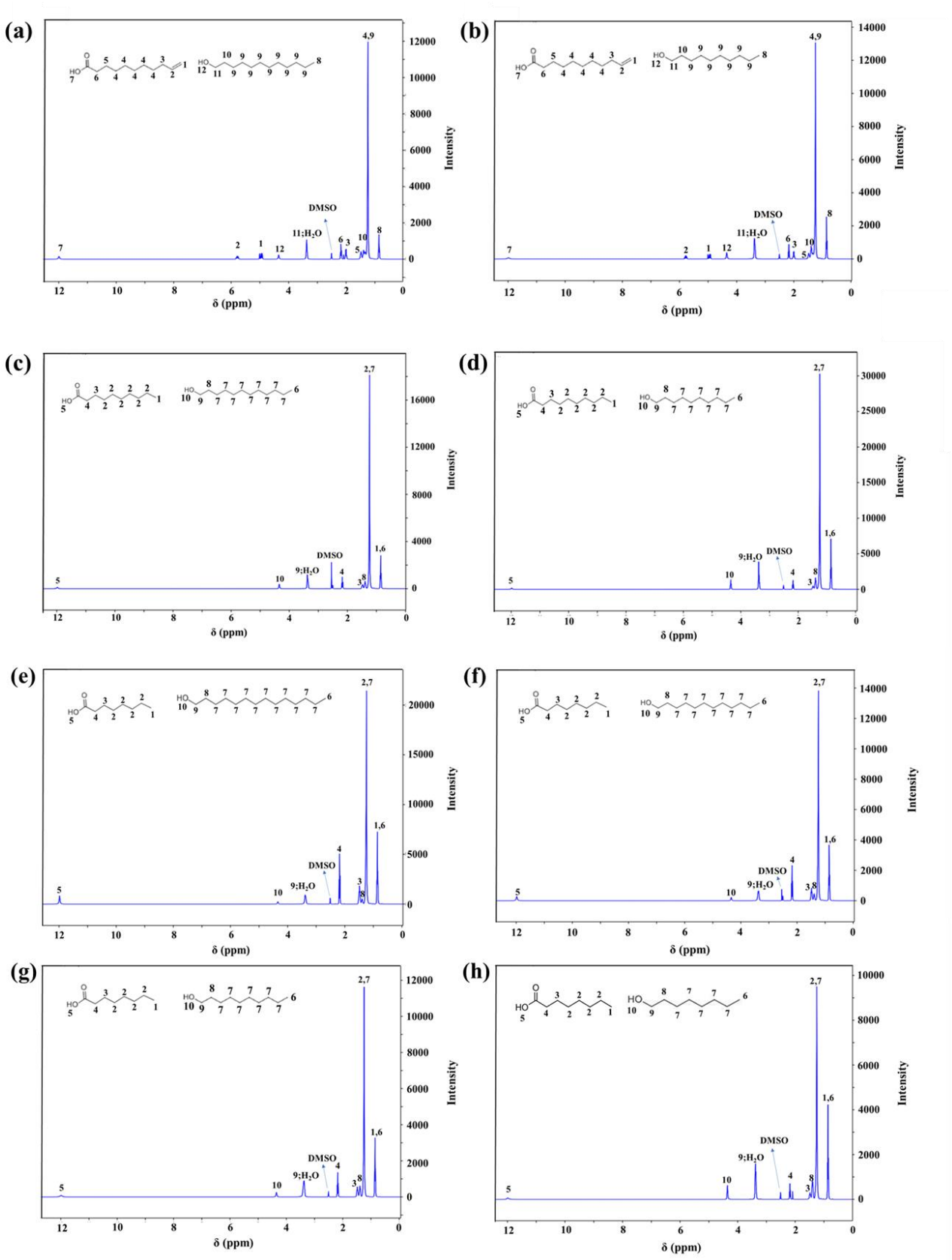


Fig. S2. ^{1}H NMR spectra of C_{12} alcohol–UA (a), C_{10} alcohol–UA (b), C_{12} alcohol–C_{10} acid (c), C_{10} alcohol–C_{10} acid (d), C_{14} alcohol–C_{8} acid (e), C_{12} alcohol–C_{8} acid (f), C_{10} alcohol–C_{8} acid (g) and C_{8} alcohol–C_{8} acid (h) HDESs. HBA–HBD mole ratio is at eutectic ratio for each HDES; UA: 10-undecenoic acid.

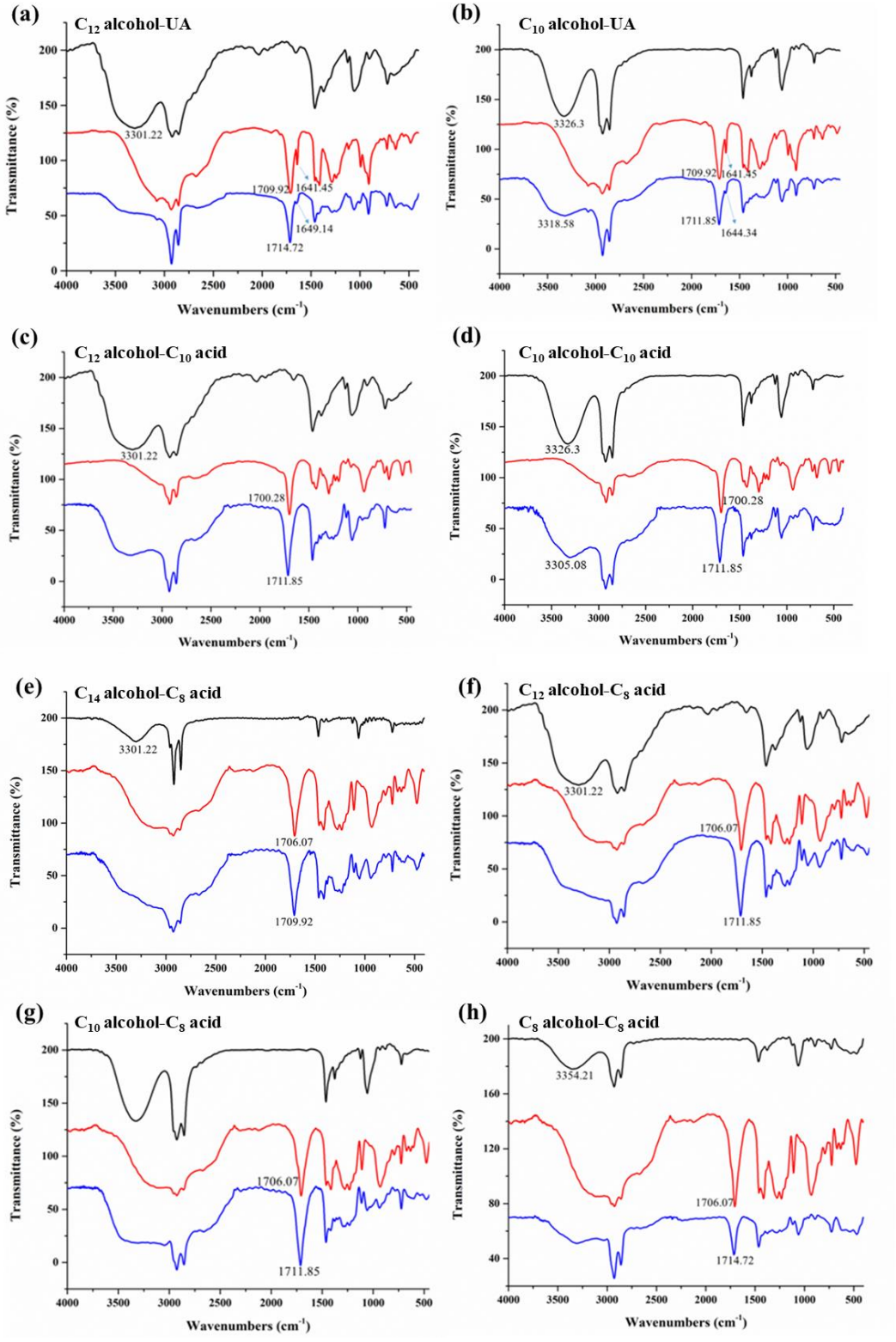


Fig. S3. FT-IR spectra of HDESs and their components (a: C_{12} alcohol–UA, b: C_{10} alcohol–UA, c: C_{12} alcohol–C_{10} acid, d: C_{10} alcohol–C_{10} acid, e: C_{14} alcohol–C_8 acid, f: C_{12} alcohol–C_8 acid, g: C_{10} alcohol–C_8 acid, h: C_8 alcohol–C_8 acid), where black lines are pure long chain alkanols, red lines represent pure long chain alkyl carboxylic acids and blue lines refer to corresponding HDESs. HBA-HBD mole ratio is at eutectic ratio for each HDES; UA: 10-undecenoic acid.

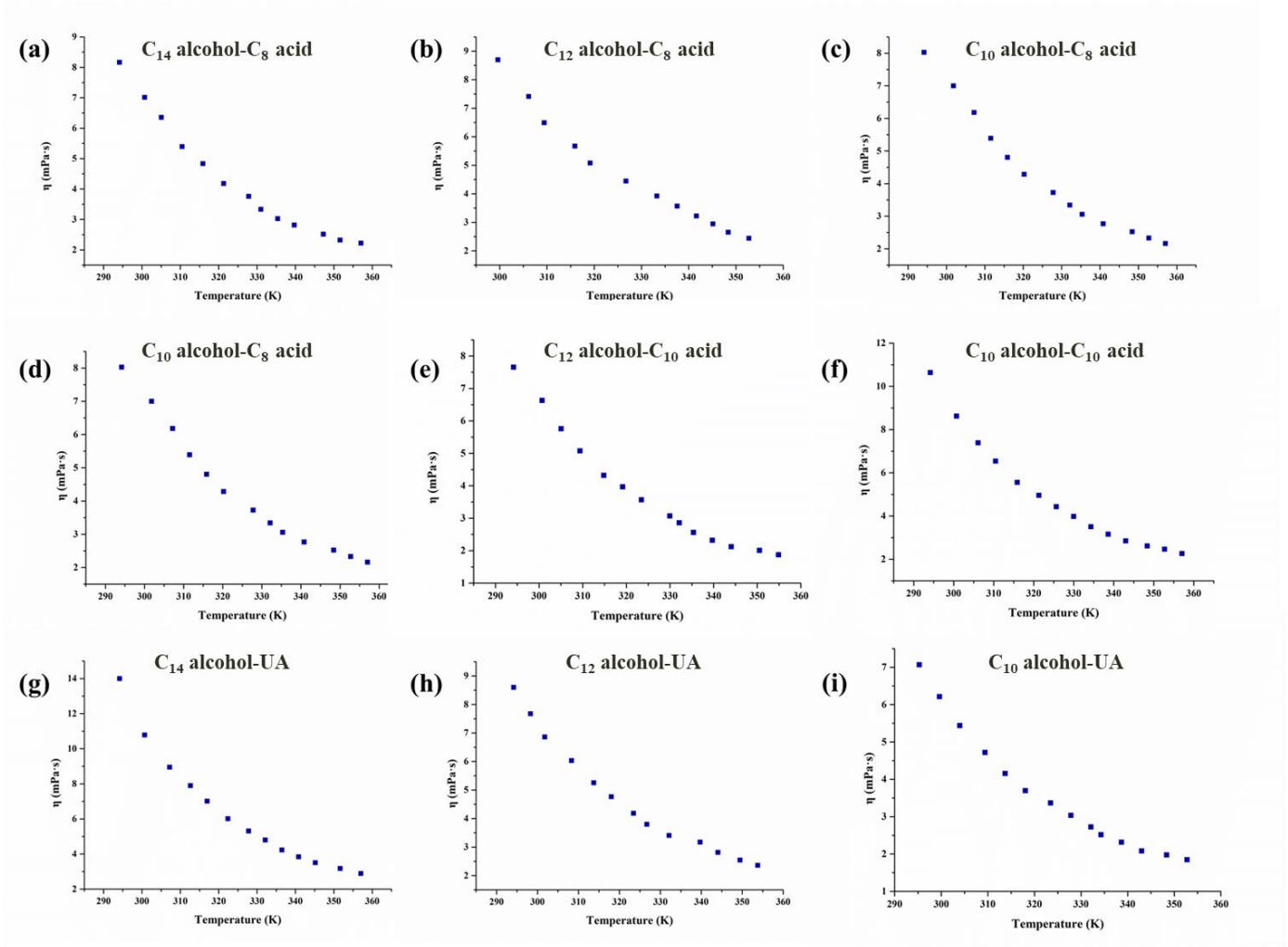


Fig. S4. Viscosities of nine HDESs as function of temperature. HBA–HBD mole ratio is at eutectic ratio for each HDES; UA: 10-undecenoic acid.

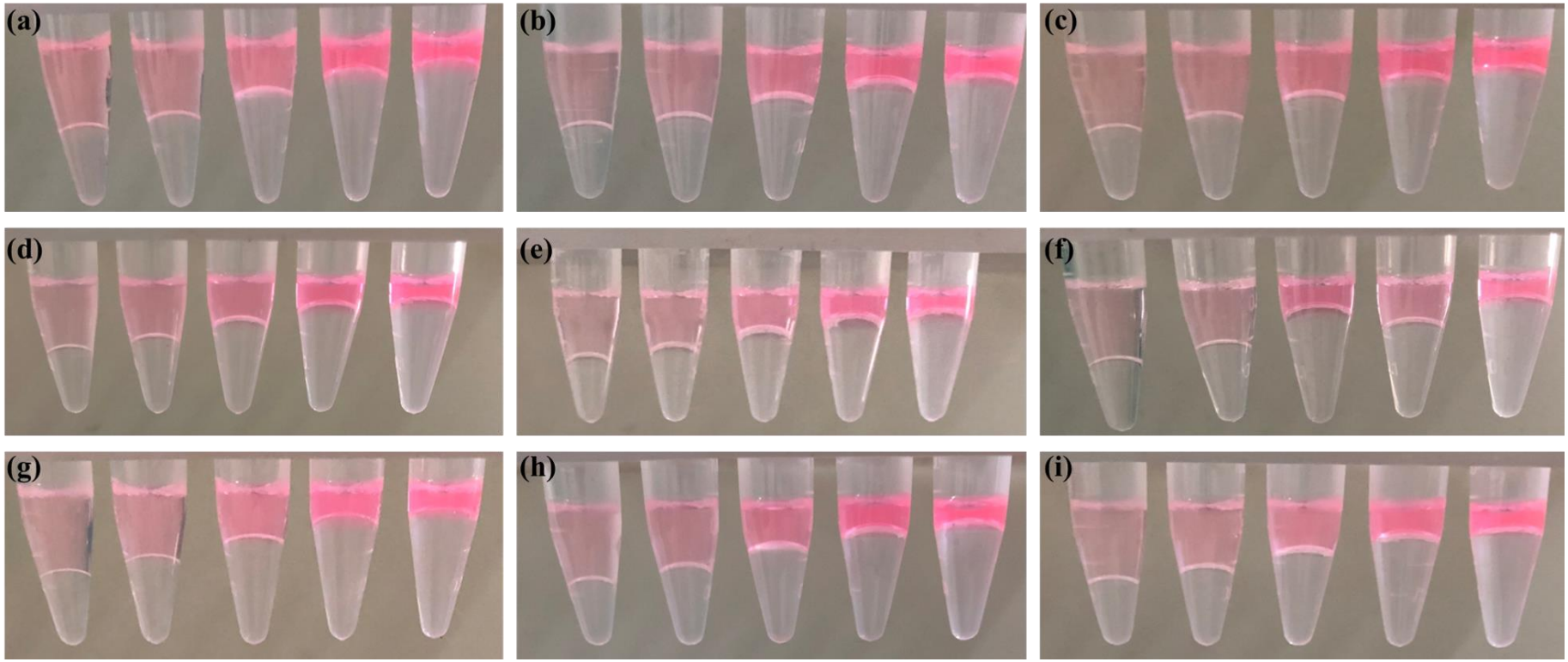


Fig. S5. The mixtures of C_{14} alcohol–C_8 acid (a), C_{12} alcohol–C_8 acid (b), C_{10} alcohol–C_8 acid (c), C_8 alcohol–C_8 acid (d), C_{12} alcohol–C_{10} acid (e), C_{10} alcohol–C_{10} acid (f), C_{14} alcohol–UA (g), C_{12} alcohol–UA (h) and C_{10} alcohol–UA (i) with water (containing 0.1 mM rhodamine B as indicator) at volume ratio of 3:1, 2:1, 1:1, 1:2 and 1:3 (from left to right) after vortex and centrifugation. HBA-HBD mole ratio is at eutectic ratio for each HDES; UA: 10-undecenoic acid.

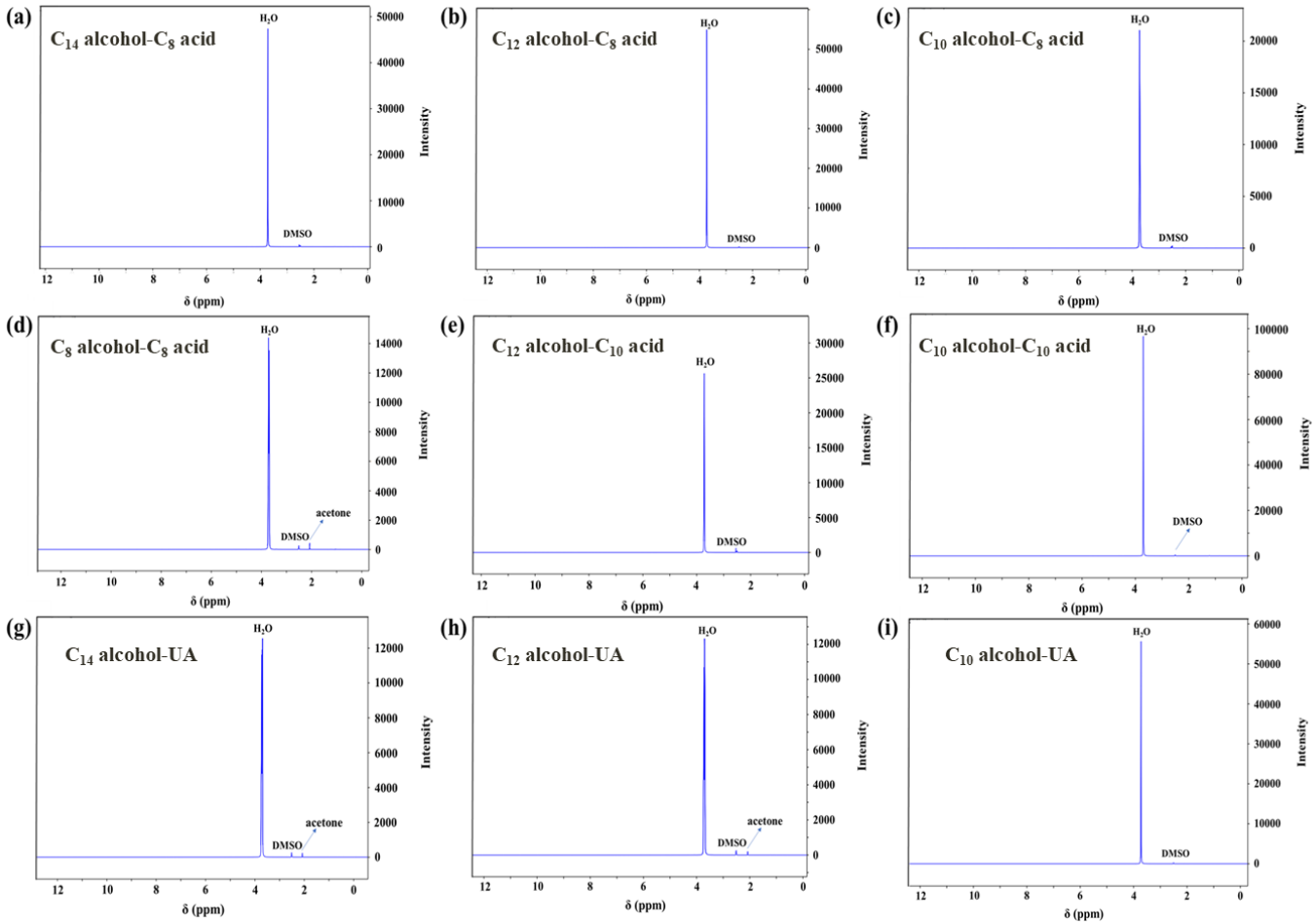


Fig. S6. ^{1}H NMR spectra of the aqueous phase obtained after HDESs being mixed with water (vortex and centrifugation). HBA–HBD mole ratio is at eutectic ratio for each HDES; UA: 10-undecenoic acid.

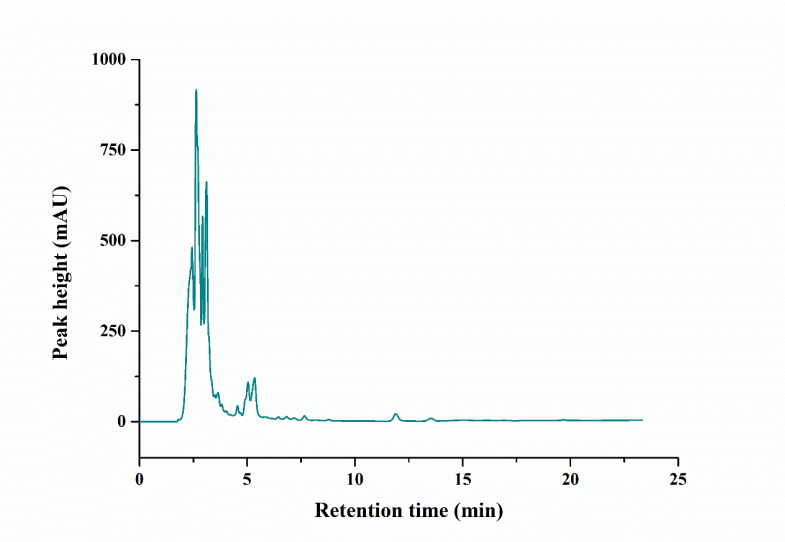


Fig. S7. The chromatogram of the aqueous phase obtained after extracting AQs from RRR sample using C_{14} alcohol–UA HDES–water two-phase system**.**

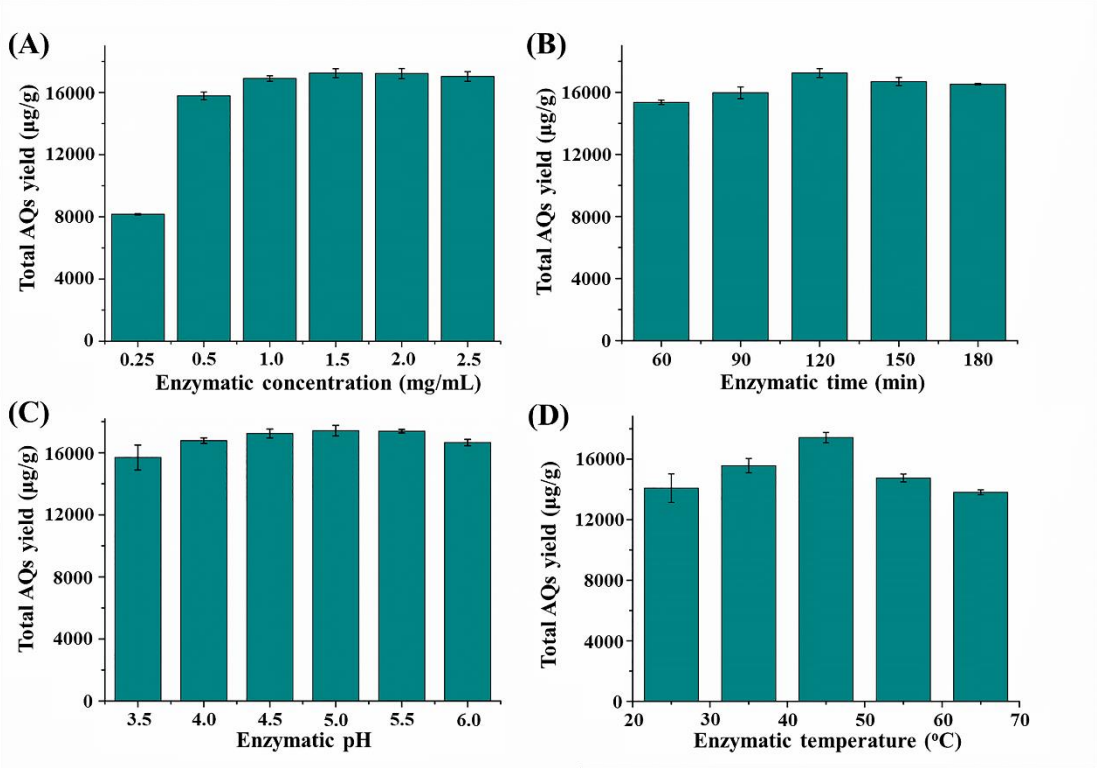


Fig. S8. Effect of enzyme concentration (a), enzymatic time (b), enzymatic pH (c), and enzymatic temperature (d) on the total AQs yield.

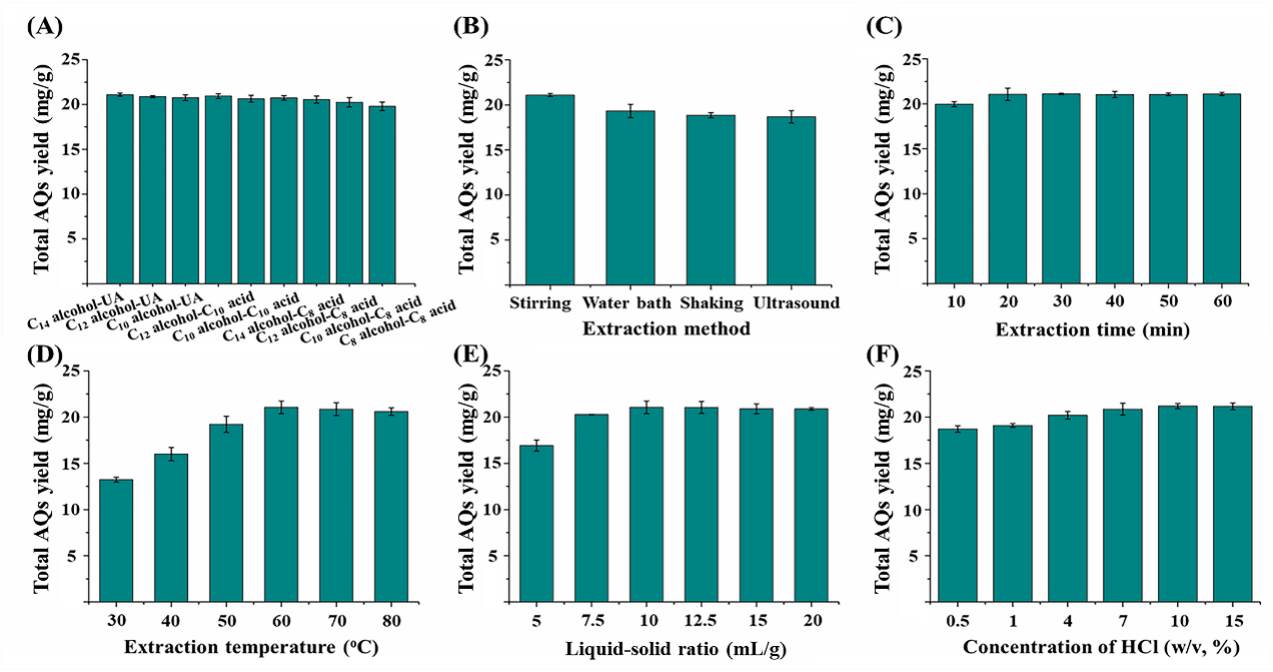


Fig. S9. Effects of type of HDESs (A), extraction method (B), extraction time (C), extraction temperature (D), liquid-solid ratio (E), and concentration of HCl (F) on the total AQs extraction yield.

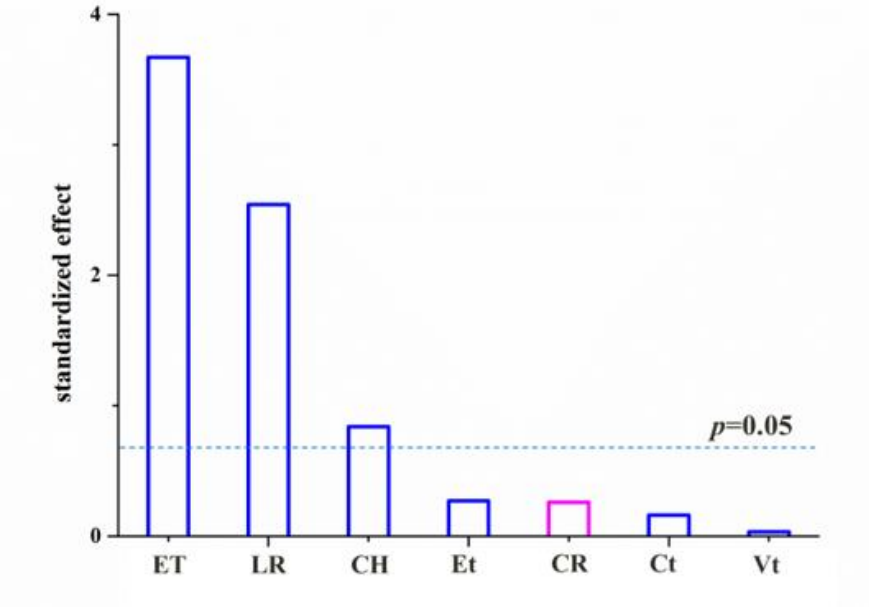


Fig. S10. Standardized Pareto chart for Plackett-Burman design. Blue framed columns, positive values; magenta framed column, negative value. The dotted line represents 95% confidence level.

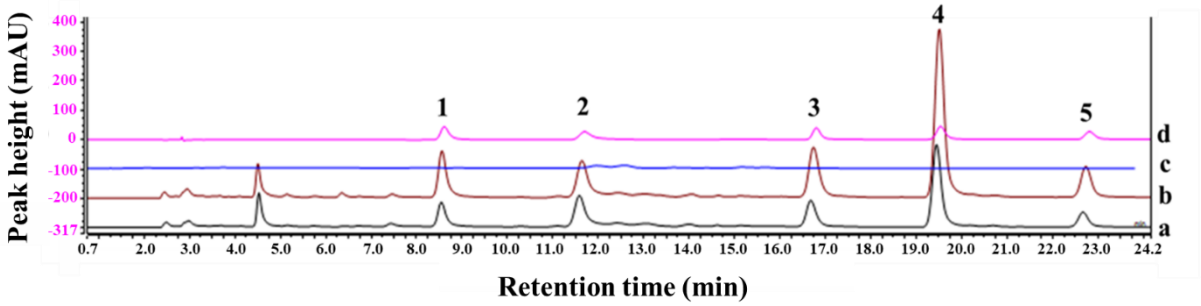
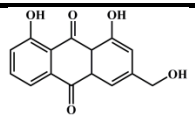
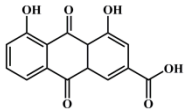
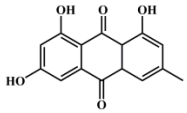
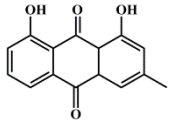
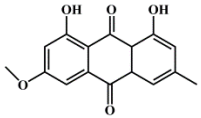


Fig. S11. Representative chromatograms: (a) the HDES-rich phase obtained after extraction of AQs from RRR sample; (b) the HDES-rich phase obtained after extraction of AQs from 50% spiked RRR sample; (c) the blank HDES phase; (d) 10 μg/mL standard AQs solution. Peak identification: 1, aloe-emodin; 2, rhein; 3, emodin; 4, chrysophanol; 5, physcion. HDES: C_{14} alcohol–UA (1:4).

4. Supplementary tables

Table S1

Chemical structures and physical properties of tested AQs.

AQ	Chemical structure	log*P*[a]	pK_a[a]	HBA[b]	HBD[c]
Aloe-emodin		3.254±0.915	6.30±0.20	5	3
Rhein		4.290±0.824	3.17±0.20	6	3
Emodin		3.641±0.951	6.39±0.20	5	3
Chrysophanol		4.720±0.824	6.63±0.20	4	2
Physcion		5.078±0.917	6.23±0.20	5	2

[a] Data obtained using SciFinder Scholar from Chemical Abstract Service;

[b] HBA = Hydrogen bond acceptor;

[c] HBD = Hydrogen bond donor.

Table S2

The initial screening result of HDESs.

	Mole ratio (HBA: HBD)	C_8 alcohol	C_{10} alcohol	C_{12} alcohol	C_{14} alcohol
C_8 acid	1:3	×	✓	✓	✓
	1:2	×	✓	✓	✓
	1:1	×	✓	✓	×
	2:1	✓	✓	✓	×
	3:1	✓	✓	✓	×
C_{10} acid	1:3	×	×	✓	○
	1:2	×	×	✓	○
	1:1	×	×	✓	○
	2:1	×	✓	✓	○
	3:1	×	✓	✓	○
10-undecylenic acid (UA)	1:3	×	×	✓	✓
	1:2	×	×	✓	✓
	1:1	×	✓	✓	✓
	2:1	×	✓	✓	×
	3:1	×	✓	✓	×

Black check mark: DES can be formed at room temperature. Red circle: DES can be formed, but it is not a liquid at room temperature.

Red cross: DES cannot be formed. Alkyl carboxylic acid is used as hydrogen bond donor (HBD), and alkanol as HBA (hydrogen bond acceptor).

Table S3

Fitted parameters of VFT formula and correlation coefficient (R^2) for HDESs.

HDES (HBA–HBD)	Mole ratio (HBA:HBD)	A_η (mPa s)	B_η (K)	C_η (K)	R^2
C_{14} alcohol–UA	1:4	-3.12	954.04	127.98	0.9985
C_{12} alcohol–UA	1:1	-3.26	1034.52	102.91	0.9974
C_{10} alcohol–UA	2:1	-3.40	898.36	127.80	0.9978
C_{14} alcohol–C_8 acid	1:4	-2.99	888.76	120.69	0.9965
C_{12} alcohol–C_8 acid	1:2	-3.68	1192.63	95.89	0.9973
C_{10} alcohol–C_8 acid	1:1	-3.86	1277.33	81.03	0.9958
C_8 alcohol–C_8 acid	3:1	-3.30	869.29	134.38	0.9964
C_{12} alcohol–C_{10} acid	3:2	-4.11	1191.76	101.36	0.9952
C_{10} alcohol–C_{10} acid	3:1	-3.91	1190.94	104.80	0.9986

VFT formula: $\ln\eta = A_\eta + \frac{B_\eta}{T-C_\eta}$, where η and T represent the viscosity (mPa·s) and temperature (K). A_η, B_η and C_η are tunable parameters, and the A_η and C_η parameters are corresponding viscosity at maximum temperature and corresponding temperature at maximum viscosity, respectively (B.D. Ribeiro, C. Florindo, L.C. Iff, et al., Menthol-based eutectic mixtures: hydrophobic low viscosity solvents, ACS Sustain. Chem. Eng. 3 (2015) 2469-2477). UA:10-undecylenic acid.

Table S4

Water content of DES-rich phase obtained after HDES being mixed with water (1:1 volume ratio).

HDES (HBA–HBD)	Mole ratio (HBA:HBD)	Water content (wt%)
C_{14} alcohol–UA	1:4	2.136±0.062
C_{12} alcohol–UA	1:1	2.841±0.092
C_{10} alcohol–UA	2:1	3.149±0.082
C_{14} alcohol–C_8 acid	1:4	3.269±0.061
C_{12} alcohol–C_8 acid	1:2	3.413±0.049
C_{10} alcohol–C_8 acid	1:1	3.679±0.026
C_8 alcohol–C_8 acid	3:1	4.413±0.052
C_{12} alcohol–C_{10} acid	3:2	3.194±0.254
C_{10} alcohol–C_{10} acid	3:1	3.208±0.061

UA: 10-undecylenic acid.

Table S5

Experimental domain for Plackett-Burman design.

Factor	Unit	Abbreviation	Low level (-1)	High level (+1)
Extraction temperature	^{o}C	ET	30	60
Extraction time	min	Et	10	30
Liquid-solid ratio	mL/g	LR	5	10
Concentration of HCl	w/v, %	CH	0.5	10
Vortex time	s	Vt	5	30
Centrifugal time	min	Ct	3	10
Centrifugal rate	rpm	CR	3000	6000

Table S6

Experimental design and response values with different combinations of extraction temperature, liquid-solid ratio and concentration of HCl in the Box-Behnken design.

Run	A: extraction temperature (^{o}C)	B: liquid-solid ratio (mL/g)	C: concentration of HCl (w/v, %)	Response: total AQs yield (mg/g)
1	55.00	10.00	7.75	21.065
2	80.00	15.00	7.75	20.290
3	80.00	10.00	0.50	18.232
4	80.00	5.00	7.75	18.463
5	30.00	15.00	7.75	14.836
6	55.00	10.00	7.75	20.446
7	80.00	10.00	15.00	20.246
8	30.00	10.00	15.00	13.864
9	55.00	5.00	0.50	16.525
10	55.00	15.00	0.50	18.018
11	55.00	15.00	15.00	20.124
12	30.00	5.00	7.75	11.880
13	55.00	5.00	15.00	17.340
14	55.00	10.00	7.75	20.665
15	30.00	10.00	0.50	13.178
16	55.00	10.00	7.75	20.432
17	55.00	10.00	7.75	20.364

Table S7

ANOVA of the established BBD model.

Source	Sum of squares	Degree of freedom	Mean square	*F* value	*p* value	Prob>*F*
Model	139.07	9	15.45	263.56	< 0.0001	significant
A	68.88	1	68.88	1174.88	< 0.0001	
B	10.25	1	10.26	175.08	< 0.0001	
C	3.95	1	3.95	67.36	< 0.0001	
AB	0.32	1	0.32	5.44	0.0525	
AC	0.44	1	0.44	7.59	0.0283	
BC	0.42	1	0.42	7.08	0.0324	
A^2	36.00	1	36.00	613.97		
B^2	7.15	1	7.15	121.96		
C^2	7.00	1	7.00	119.31		
Residual	0.41	7	0.059			
Lack of fit	0.082	3	0.027	0.34	0.8019	not significant
Pure error	0.33	4	0.082			
Cor total	139.48	16				
	R^2=0.9971	Adjusted R^2=0.9933		Predicted R^2=0.9869		

Table S8

The standard curve and method validation for HPLC-DAD analysis of 1,8-dihydroxyanthraquinone.

Analyte	Added (μg/mL)	Intra-day (n=6)		Inter-day (n=3)		Linear equation (n=8)	Linear range (μg/mL)	R	LOD (μg/mL)	LOQ (μg/mL)
		Recovery (%)	RSD (%)	Recovery (%)	RSD (%)					
1,8-dihydroxyanthraquinone	5	99.9	0.8	98.1	3.9	y=63866x-2216	5-100	0.9998	0.0132	0.0440
	30	100.5	0.5	100.7	1.2					
	100	101.1	0.15	100.8	0.4					

Table S9

Analytical performance for five AQs analysis by C_{14} alcohol–UA DES based extraction method with HPLC-DAD.

Analyte	Linearity equation (n=8)	Linear range (μg/mL)	R	LOD (μg/mL)	LOQ (μg/mL)	Intra-day RSD (%) (n=6)	Inter-day RSD (%) (n=3)	Spiked level (mg/g)	Recovery (%) (n=3)	RSD (%)
Aloe emodin	y = (0.9684±0.0122)x+ (0.0107±0.0318)	0.15-40	0.9997	0.010	0.033	2.37	3.75	3.57	97.3	1.37
								2.38	98.1	0.68
								1.19	99.6	3.36
Rhein	y = (0.7721±0.0182)x- (0.0024±0.0777)	0.15-40	1	0.018	0.059	1.01	1.11	6.65	87.1	1.76
								4.43	86.9	3.53
								2.22	88.6	2.11
Emodin	y = (0.7497±0.0037)x+ (0.0257±0.0249)	0.15-40	0.9997	0.010	0.033	0.57	1.62	5.65	101.2	2.43
								3.76	99.4	1.71
								1.88	99.0	1.20
Chrysophanol	y = (1.0060±0.0078)x+ (0.0280±0.0611)	0.15-100	0.9999	0.008	0.028	0.65	1.20	12.68	102.3	2.79
								8.45	102.6	1.02
								4.22	98.9	3.84
Physcion	y = (0.6956±0.0110)x- (0.0123±0.0181)	0.15-30	0.9999	0.015	0.049	1.60	2.15	3.74	100.7	0.73
								2.49	99.1	2.76
								1.25	99.5	1.12

Table S10

The stability validation for five AQs analysis by C_{14} alcohol–UA DES based extraction method with HPLC-DAD.

Analyte	Initial content (mg/g)	RSD (%) (n=3)	Content after 24 h (mg/g)	RSD (%) (n=3)	Relative error (%)
Aloe-emodin	2.41	1.6	2.30	1.7	-4.6
Rhein	4.44	3.5	4.35	1.2	-2.0
Emodin	3.89	0.2	3.78	0.3	-2.8
Chrysophanol	8.42	1.9	8.47	3.4	0.6
Physcion	2.52	1.7	2.46	2.6	-2.4