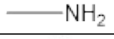
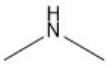
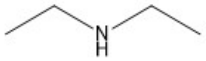
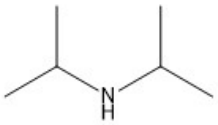
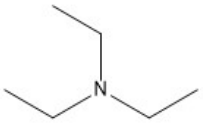
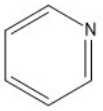
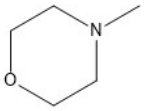
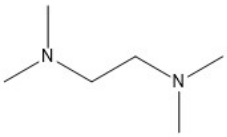
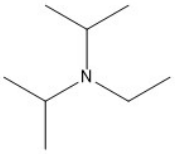
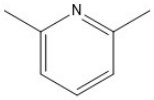
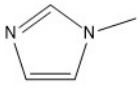
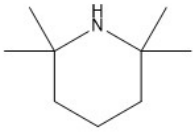
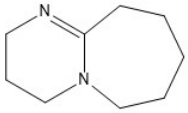
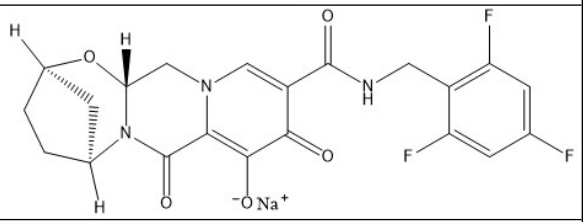
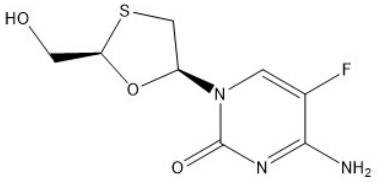
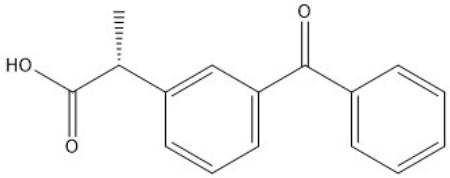
Electronic Supplementary Material (ESI) for Analytical Methods.

Supplemental Information

Table S1 Common names, abbreviations, and structures of volatile amines, APIs, and passivation reagent evaluated.

Category	Name (Abbreviation)	Structure
Volatile amine	Methylamine (MA)*	
	Dimethylamine (DMA)	
	t-Butylamine (TBA)	
	Diethylamine (DEA)	
	Butylamine (BA)	
	Diisopropylamine (DIPA)	
	Pyrrolidine (PYR)	
	Triethylamine (TEA)	
	Pyridine (PY)	
	N-Methylmorpholine (NMM)	
	Tetramethylethylnediamine (TMEDA)	
	N, N-Diisopropylethylamine (DIPEA)	
	2,6-Lutidine (26L)	
	N-methylimidazole (NMI)	

	2,2,6,6-tetramethylpiperidine (TMP)	
Passivation reagent	1,8-diazabicyclo [5.4.0] undec-7-ene (DBU)	
Active pharmaceutical ingredient (API)	Bictegravir Sodium (BIC)	
	Emtricitabine (FTC)	
	Ketoprofen® (KET)	

*Controlled substance; only used to evaluate separation specificity due to limited availability.

Table S2 Headspace sampler and GC parameters selected for the analysis of volatile amines.

GC parameters	
Gas Chromatography System	Agilent 7890
Chromatographic Column	Rtx-Volatile Amine 30 m x 0.32 mm, 5.0 µm
Carrier Gas	Helium
Carrier Flow	Constant Flow at 2.0 mL/min
Injection Mode	Split 10:1 (EPC)
Inlet Temperature	220 °C
Oven Temperature Program	40 °C initial temperature, hold for 2 minutes Ramp at 8 °C/min to 70 °C, no hold Ramp at 5 °C/min to 100 °C, no hold Ramp at 10 °C/min to 200 °C, no hold Ramp at 30 °C/min to 230 °C, hold for 7.2 minutes
Detection Method	Flame Ionization Detection
Detector Gas Flow Rate	Fuel (Hydrogen): 40 mL/min
Detector Temperature	270 °C
Run Time	30 min
Headspace Sampler Parameters	
Oven Temperature	120°C
Loop Temperature	130 °C
Transfer Line Temperature	180 °C
GC Cycle Time	37 min
Vial Equilibration Time	10 min
Vial Pressurization Time	0.2 min
Injection Time	1.0 min
Vial Pressurization Mode	Fill to pressure, 20 psi
Loop Fill Mode	Default
Vent after Extraction	Yes
Vial Size	20 mL
Shaking Level	9
Loop Volume	1.0 mL
Vial Standby Flow	10 L/min

Table S3 Comparison of the recovery of volatile amines from BIC API dissolved in DMAc containing various concentrations of DBU.

Volatile Amine	Pure DMAc		0.1% (v/v) DBU/DMAc		5% (v/v) DBU/DMAc		10% (v/v) DBU/DMAc	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DMA	90.0	0.6	87.7	1.4	80.6	0.9	78.2	0.9
TBA	99.6	0.8	96.8	0.8	94.6	1.3	97.6	0.6
DEA	98.2	1.0	93.4	2.0	96.1	2.4	103.4	0.9
BA	97.4	0.8	89.6	0.8	83.5	2.2	82.8	1.4
DIPA	106.9	1.0	103.8	1.1	102.8	1.8	109.5	0.7
PYR	98.2	1.0	89.6	0.8	80.8	0.8	75.4	0.4
TEA	108.9	1.3	104.2	1.2	100.5	1.1	103.3	0.6
PY	112.5	0.8	101.2	1.6	95.5	3.1	101.8	0.9
NMM	110.7	0.9	100.5	1.3	96.4	2.5	100.9	0.4
TMEDA	103.5	1.2	96.8	1.2	101.2	1.8	113.4	0.5
DIPEA	108.0	0.6	99.1	0.9	97.7	0.4	97.7	1.9
26L	110.6	3.8	95.5	3.5	90.7	4.8	98.4	0.8
NMI	93.4	5.8	81.2	8.2	65.9	6.7	72.7	5.8
TMP	115.9	2.1	100.9	1.5	99.6	3.1	104.6	0.8

Table S4 Comparison of the recovery of volatile amines from FTC API dissolved in DMAc containing various concentrations of DBU.

Volatile Amine	Pure DMAc		0.1% (v/v) DBU/DMAc		5% (v/v) DBU/DMAc		10% (v/v) DBU/DMAc	
	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)
DMA	79.5	1.6	58.2	0.7	62.6	1.6	55.3	0.6
TBA	83.8	1.9	88.6	0.8	90.8	1.8	92.9	0.6
DEA	94.0	1.3	91.8	0.9	94.5	1.7	92.0	0.7
BA	40.5	5.1	27.0	1.8	36.6	1.9	33.1	1.0
DIPA	118.2	1.8	118.9	0.4	111.5	2.3	113.7	0.5
PYR	45.9	2.8	23.2	1.6	23.6	1.5	18.6	1.5
TEA	113.2	1.8	104.8	1.0	105.7	2.8	109.0	0.6
PY	117.0	1.2	101.8	1.9	103.8	3.8	100.7	1.0
NMM	114.7	1.7	97.2	1.9	95.6	2.7	99.7	0.4
TMDEA	104.3	1.3	103.8	1.6	109.8	0.9	97.4	0.9
DIPEA	117.5	2.9	100.7	1.1	102.8	0.6	105.9	1.6
26L	117.1	1.7	94.1	2.9	92.0	4.4	97.0	3.4
NMI	105.9	1.7	85.2	8.6	69.0	3.3	76.7	2.9
TMP	116.4	1.9	106.0	2.3	106.5	2.9	112.8	0.7

Table S5 Precision of LOQ injections (n=6) following system passivation with and without 5% (v/v) DBU/DMAc.

Analyte	System passivation using DMAc (%RSD)	System passivation using 5% (v/v) DBU/DMAc (%RSD)
DMA	0.9	0.8
TBA	1.3	0.5
DEA	4.6	0.8
BA	2.8	1.0
DIPA	1.9	1.0
PYR	5.3	1.3
TEA	2.3	0.9
PY	3.6	1.8
NMM	2.5	1.5
TMDEA	2.8	1.2
DIPEA	2.1	0.8
26L	4.4	1.2
NMI	11.2	5.9
TMP	3.1	1.3

Table S6 Specificity and resolution of volatile amines in the presence of common residual solvents used in API manufacturing.

Analyte	5% (v/v) DBU/DMAc		5% (v/v) DBU/NMP		5% (v/v) DBU/DMAc	5% (v/v) DBU/NMP
	Retention Time (min)	Relative retention time to DBU	Retention Time (min)	Relative retention time to DBU	USP Resolution	
MA	2.35	0.09	2.63	0.10	NA	NA
MeOH	2.66	0.10	2.95	0.11	3.0	3.4
DMA	3.12	0.12	3.47	0.13	4.2	5.1
EtOH	3.72	0.14	4.09	0.15	5.3	5.9
ACN	4.05	0.16	4.45	0.17	2.8	3.4
Acetone	4.37	0.17	4.79	0.18	2.8	3.1
IPA	4.59	0.18	5.02	0.19	1.8	1.9
TBA	5.10	0.20	5.56	0.21	3.4	4.0
***t*-Butanol**	5.28	0.20	5.78	0.22	1.2	1.3
DCM	5.47	0.21	5.95	0.22	1.4	1.8
DEA/MTBE	6.604/6.689	0.23/0.26	7.15	0.27	6.7	8.0
MEK	7.01	0.27	7.56	0.28	-	2.6
2-Butanol	7.30	0.28	7.85	0.29	2.1	2.3
Diisopropyl Ether/ EtOAc	7.66	0.29	8.23	0.31	1.6	2.2
***n*-Hexane**	7.76	0.30	8.35	0.31	0.4	0.6
THF	8.31	0.32	8.93	0.33	3.5	4.0
BA	8.47	0.33	9.10	0.34	1.2	1.3
IPAc/ *n*-Butanol	9.22	0.35	9.85	0.37	5.0	5.4
DIPA	9.52	0.37	10.19	0.38	-	1.8
Benzene	9.60	0.37	10.19	0.38	-	-
2-Methyl THF	9.75	0.37	10.43	0.39	-	1.3
PYR	10.09	0.39	10.80	0.40	2.3	2.7
TEA	10.47	0.40	11.15	0.42	2.3	2.3
1,4-Dioxane	10.76	0.41	11.46	0.43	1.8	1.9
***n*-Heptane**	11.12	0.43	11.82	0.44	2.5	2.5
MIBK	11.89	0.46	12.58	0.47	4.9	5.4
PY	12.02	0.46	12.72	0.47	0.8	1.2
DMF	12.62	0.48	13.27	0.49	3.9	4.8
Toluene	13.27	0.51	13.93	0.52	4.5	5.6
NMM	13.67	0.53	14.32	0.53	3.1	3.2
TMEDA	14.50	0.56	15.12	0.56	6.3	6.3
DIPEA	14.92	0.57	15.54	0.58	3.0	3.1
26L*	16.26	0.62	—	—	1.3	N/A
NMI	16.69	0.64	17.30	0.64	4.4	10.6
TMP	17.95	0.69	18.52	0.69	11.1	7.9
DBU	26.02	1.00	26.84	1.00	-	-

* 26L is not evaluated for specificity in 5% (v/v) DBU/NMP due to the presence of interfering artifact peak from stemming from NMP.

Table S7 Analytical validation results for the analysis of volatile amines in 5% (v/v) DBU/DMAc or 5% (v/v) DBU/NMP

Analyte	LOD† (mg/mL)	LOQ‡ (mg/mL)	Diluent: 5 % (v/v) DBU/DMAc			Diluent: 5% (v/v) DBU/NMP		
			LOQ RSD (%) (n=6)	Linearity Range (mg/mL)	Correlation Coefficient (R^2)	LOQ RSD (%) (n=6)	Linearity Range (mg/mL)	Correlation Coefficient (R^2)
DMA	0.025	0.05	0.8	0.05-2.5	1.000	1.7	0.05-2.5	1.000
TBA	0.005	0.01	0.8	0.01-2.5	1.000	0.7	0.01-2.5	1.000
DEA	0.0025	0.005	1.2	0.005-2.5	1.000	1.5	0.005-2.5	1.000
BA	0.005	0.01	0.3	0.01-2.5	1.000	0.7	0.01-2.5	1.000
DIPA	0.005	0.01	0.6	0.01-2.5	1.000	1.2	0.01-2.5	1.000
PYR	0.025	0.05	0.4	0.05-2.5	1.000	0.6	0.05-2.5	1.000
TEA	0.005	0.01	0.6	0.01-2.5	0.999	1.2	0.01-2.5	1.000
PY	0.005	0.01	0.7	0.01-2.5	1.000	0.8	0.01-2.5	1.000
NMM	0.005	0.01	0.3	0.01-2.5	1.000	0.9	0.01-2.5	1.000
TMEDA	0.005	0.01	0.3	0.01-2.5	1.000	1.4	0.01-2.5	1.000
DIPEA	0.005	0.01	0.3	0.01-2.5	0.999	1.7	0.01-2.5	1.000
26L*	0.005	0.01	3.0	0.01-2.5	1.000	N/A	N/A	N/A
NMI	0.025	0.05	2.2	0.05-2.5	1.000	3.1	0.05-2.5	0.999
TMP	0.005	0.01	0.6	0.01-2.5	1.000	1.7	0.01-2.5	1.000

* 26L is not evaluated for specificity in 5% (v/v) DBU/NMP due to the presence of interfering artifact peak stemming from NMP.
† LOD is inferred as 0.5x LOQ.
‡ LOQ is established based on a minimum S/N ratio of 10x.

Table S8 Solution stability results for the analysis of volatile amines in 5% (v/v) DBU/DMAc or 5% (v/v) DBU/NMP

	Recovery Range (%)			
Analyte	**Working Standard Solution:** The working standard solution is stable for up to 8 days in 5% (v/v) DBU/DMAc and 7 days in 5% (v/v) DBU/NMP when stored at room temperature.		**LOQ Solution:** The LOQ solution is stable up to 6 days for DMA and 7 days for all other volatile amines in 5% (v/v) DBU/DMAc and 5% (v/v) DBU/NMP when stored at room temperature.	
	5 % (v/v) DBU/DMAc*	**5% (v/v) DBU/NMP****	**5 % (v/v) DBU/DMAc*****	**5% (v/v) DBU/NMP****
DMA	89 - 100	96-101	71 -91	85-92
TBA	96 -102	97-99	95-98	95-98
DEA	96 - 103	97-99	100-104	96-99
BA	96 - 100	96-98	89-96	91-94
DIPA	97 - 102	97-99	100-103	103-107
PYR	91- 98	96-102	82-94	83-90
TEA	96 - 102	97-99	98-101	96-100
PY	98 - 103	96-98	97-103	99-102
NMM	98 - 102	97-98	97-104	97-100
TMEDA	97 - 101	97-98	97-104	95-99
DIPEA	96 - 101	96-98	102-112	93-97
26L†	98 - 102	NA	91-99	NA
NMI	108- 113	95-99	85-117	81-85
TMP	97 - 100	95-98	95-105	98-101

* Recovery ranges obtained by performing recovery studies of volatile amine standard at T = 0, 6, and 8 days.

** Recovery ranges obtained by performing recovery studies of volatile amine standard at T = 0, 2, 5, and 7 days.

*** Recovery ranges obtained by performing recovery studies of volatile amine standard at T = 0, 2, 6, and 8 days.

† 26L is not evaluated for specificity in 5% (v/v) DBU/NMP due to the presence of interfering artifact peak stemming from NMP.

Figure S1 Chromatogram of 0.1 mg/mL 14 amines in 5% (v/v) DBU/DMAc: (1) DMA, (2) TBA, (3) DEA, (4) BA, (5) DIPA, (6) PYR, (7) TEA, (8) PY, (9) NMM, (10) TMEDA, (11) DIPEA, (12) DMAc, (13) 26L, (14) NMI, (15) TMP, (16) DBU

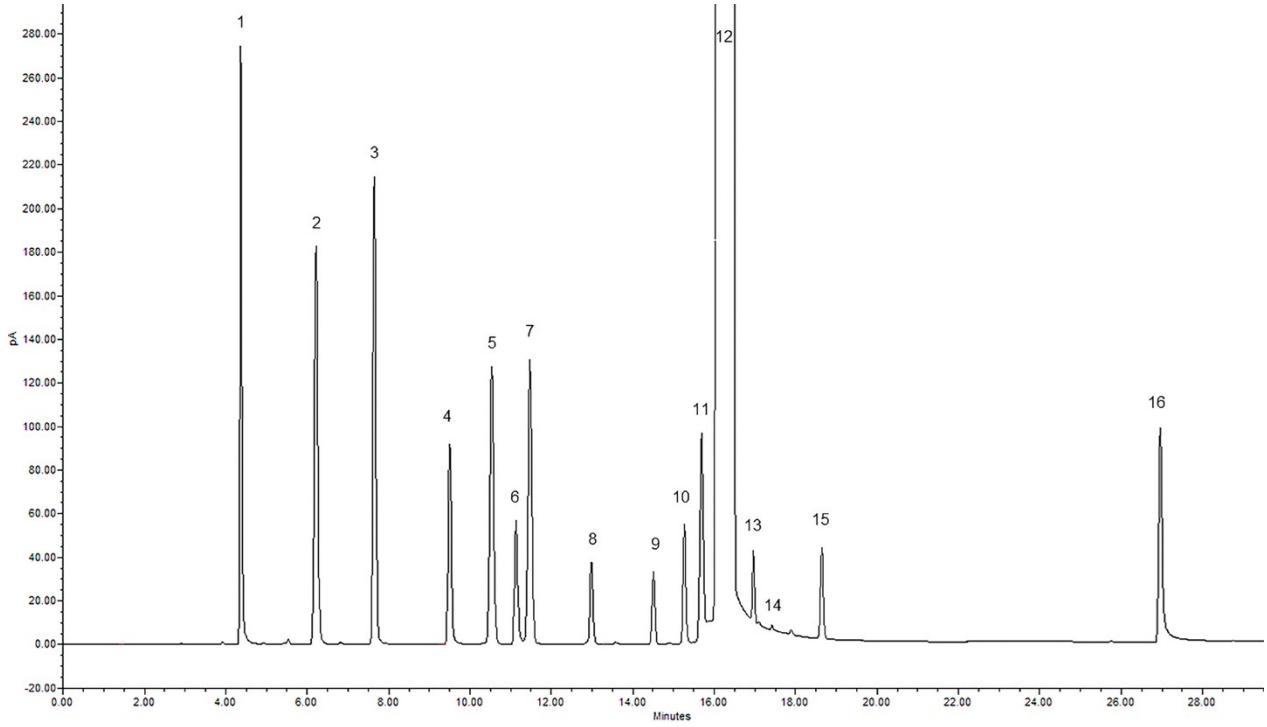


Figure S2 Comparison of the change in peak area response of LOQ solutions prepared in different DBU/DMAc diluent systems. (■) Pure DMAc, (■) 0.1% (v/v) DBU/DMAc, (■) 5% (v/v) DBU/DMAc, and (■) 10% (v/v) DBU/DMAc. *DMA peak area scaled down by 3x due to high detector response.

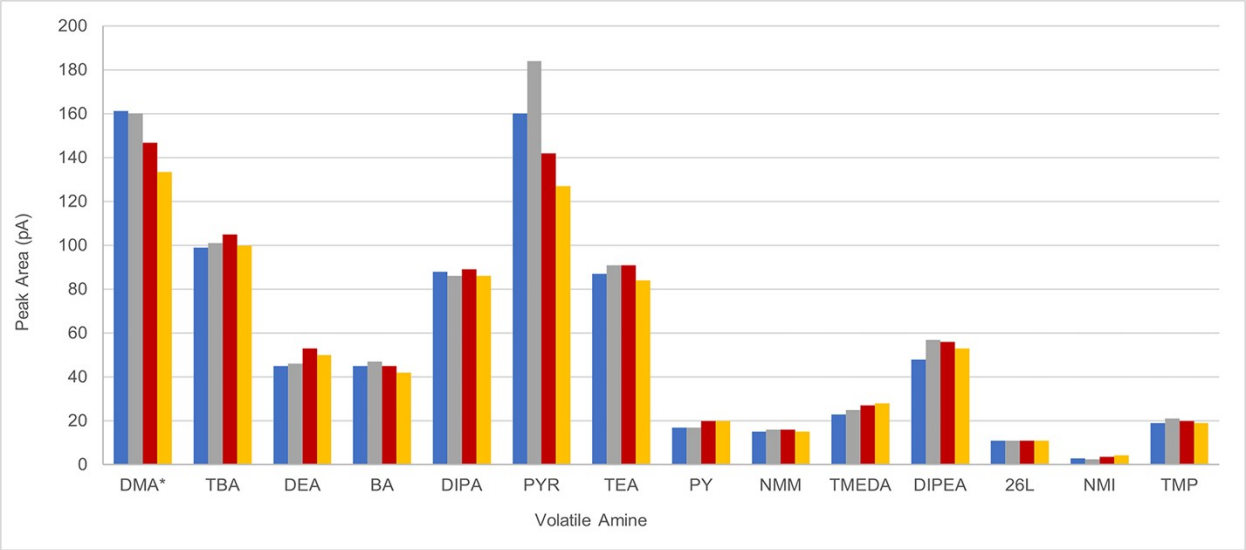


Figure S3 Chromatogram overlay of (A) 0.01N NaOH in DMAc (B) 0.5N NaOH in DMAc (C) 0.01 mg/mL 15 amines in 0.5N NaOH in DMAc (D) 0.1 mg/mL 15 amines in 0.5N NaOH in DMAc: (1) MA (2) DMA, (3) TBA, (4) DEA, (5) BA, (6) DIPA, (7) PYR, (8) TEA, (9) PY, (10) NMM, (11) TMEDA, (12) DIPEA, (13) DMAc, (14) 26L, (15) NMI, (16) TMP.

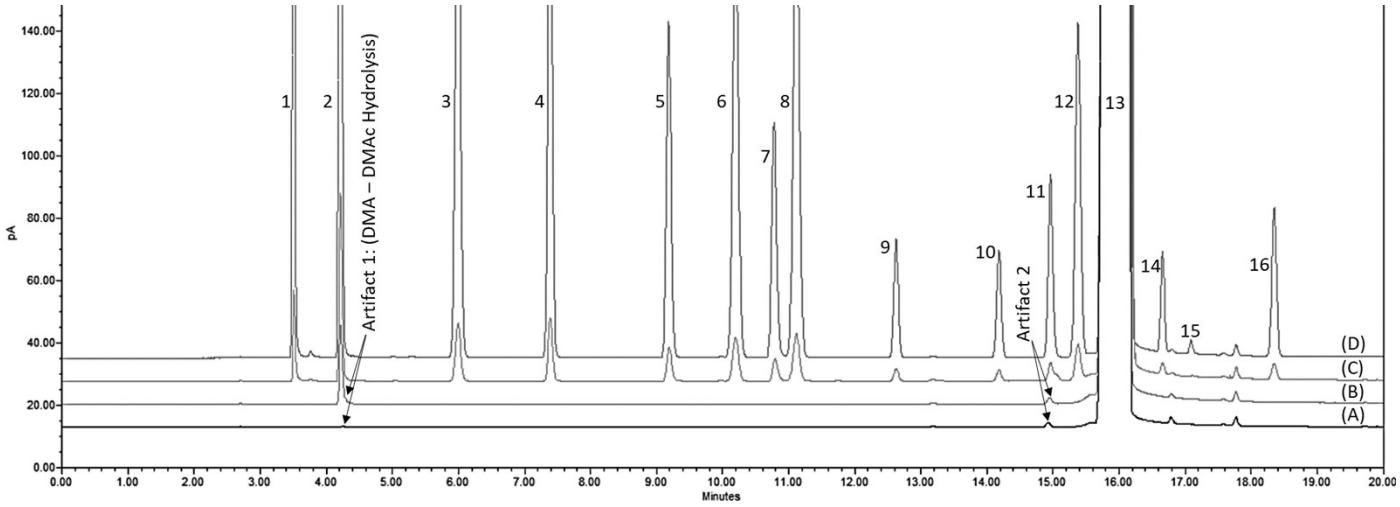


Figure S4 Chromatogram of a 0.1 mg/mL composite solution containing volatile amines and common residual solvents in 5% (v/v) DBU/DMAc. (1) MA, (2) MeOH, (3) DMA, (4) EtOH, (5) ACN, (6) Acetone, (7) IPA, (8) TBA (9) *t*-Butanol , (10) DCM, (11) DEA, (12) MTBE, (13) MEK, (14) 2-Butanol, (15) Diisopropyl ether, (16) EtOAc, (17) *n*-Hexane, (18) THF, (19) BA, (20) IPAc, (21) *n*-Butanol, (22) DIPA, (23) Benzene, (24) 2-Methyltetrahydrofuran, (25) PYR, (26) TEA, (27) 1,4-Dioxane, (28) *n*-Heptane, (29) MIBK, (30) PY, (31) DMF, (32) Toluene, (33) NMM, (34) TMEDA, (35) DIPEA, (36) DMAc, (37) 26L, (38) NMI, (39) TMP, (40) DBU.

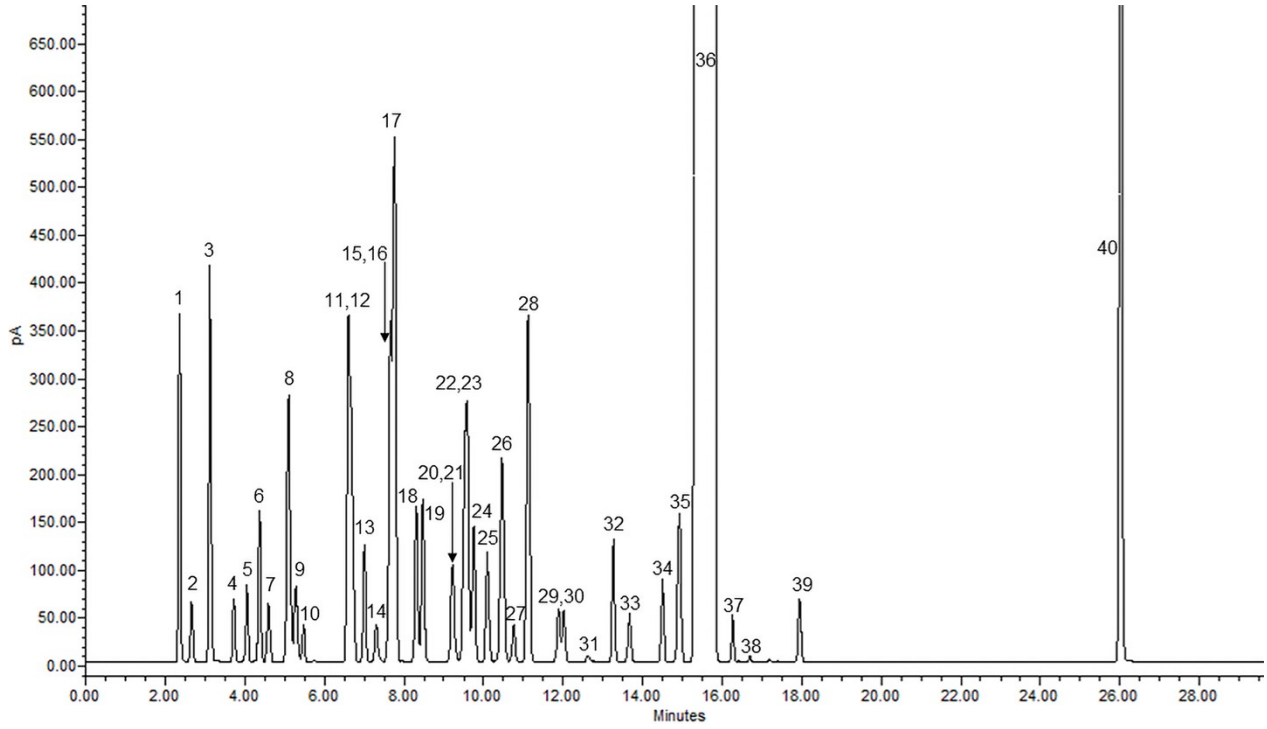


Figure S5 Chromatogram of a 0.1 mg/mL composite solution containing volatile amines and common residual solvents in 5% (v/v) DBU/NMP. (1) MA, (2) MeOH, (3) DMA, (4) EtOH, (5) ACN, (6) Acetone, (7) IPA, (8) TBA, (9) *t*-Butanol, (10) DCM, (11) DEA, (12) MTBE, (13) MEK, (14) 2-Butanol, (15) Diisopropyl Ether, (16) EtOAc, (17) *n*-Hexane, (18) THF, (19) BA, (20) IPAc, (21) *n*-Butanol, (22) DIPA, (23) Benzene, (24) 2-Methyltetrahydrofuran, (25) PYR, (26) TEA, (27) 1,4-Dioxane, (28) *n*-Heptane, (29) MIBK, (30) PY, (31) DMF, (32) Toluene, (33) NMM, (34) TMEDA, (35) DIPEA, (36) NMI, (37) TMP, (38) NMP, (39) DBU.