

1 **Developing an improved UPLC method for impurity profile analysis of**
2 **ceftriaxone using analytical quality by design**

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25 # ChaoQiang Xiao and Xia Zhang contributed equally.

26 **Tables**27 **Table S1.** Columns used in the study

Brand	Type	Packing	Specifications	L/dp
Waters	Cortecs,	C18	100 × 2.1 mm, 1.6 μm	62500
	Acquity UPLC BEH,	C18	50 × 2.1 mm, 1.7 μm	29412
	Acquity UPLC HSS ,	C18	100 × 2.1 mm, 1.8 μm	55556
Thermo	HYPERSIL GOLD	C18	100 × 2.1 mm, 1.9 μm	52632
	HYPERSIL GOLD AQ	C18	100 × 2.1 mm, 1.9 μm	52632
Shiseido	Capcell Pak C18 MGII	C18	250 × 4.6 mm, 5 μm	50000

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30 **Table S2.** Screening test operation table and response results

Exp No	A [mol/l]	B	C [°C]	D [ml/min]	E [%]	Rs1	Rs2	Rs3
1	0.01	5.0	25.0	0.384	32	1.96	1.73	0.00
2	0.05	5.0	25.0	0.384	22	13.3	4.40	1.50
3	0.01	8.0	25.0	0.384	22	3.30	0.00	0.00
4	0.05	8.0	25.0	0.384	32	3.00	2.50	1.20
5	0.01	5.0	40.0	0.384	22	11.6	1.30	0.00
6	0.05	5.0	40.0	0.384	32	5.80	1.50	0.00
7	0.01	8.0	40.0	0.384	32	0.00	0.00	0.00
8	0.05	8.0	40.0	0.384	22	5.10	4.20	2.70
9	0.01	5.0	25.0	0.713	22	9.60	0.00	0.00
10	0.05	5.0	25.0	0.713	32	4.60	1.80	0.00
11	0.01	8.0	25.0	0.713	32	0.00	0.00	0.00
12	0.05	8.0	25.0	0.713	22	3.60	7.40	2.50
13	0.01	5.0	40.0	0.713	32	3.17	1.02	0.00
14	0.05	5.0	40.0	0.713	22	2.90	13.5	1.60
15	0.01	8.0	40.0	0.713	22	4.50	0.00	0.00
16	0.05	8.0	40.0	0.713	32	2.00	2.20	1.20
17	0.03	6.5	32.5	0.548	27	4.30	0.00	0.00
18	0.03	6.5	32.5	0.548	27	4.30	0.00	0.00
19	0.03	6.5	32.5	0.548	27	4.30	0.00	0.00

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34 **Table S3.** Optimization test operation table and response results

Exp No	A	B	E [%]	Rs1	Rs2	Rs3
1	0.01	5.0	25.0	6.80	1.00	0.00
2	0.05	5.0	25.0	11.5	3.20	1.50
3	0.01	8.0	25.0	3.00	0.00	0.10
4	0.05	8.0	25.0	7.00	8.40	2.70
5	0.01	5.0	32.0	2.80	0.00	0.00
6	0.05	5.0	32.0	7.30	2.70	0.60
7	0.01	8.0	32.0	1.30	0.00	0.00
8	0.05	8.0	32.0	3.40	2.30	1.40
9	0.01	6.5	28.5	3.80	0.00	0.00
10	0.05	6.5	28.5	7.80	1.50	1.70
11	0.03	5.0	28.5	6.20	1.70	0.00
12	0.03	8.0	28.5	3.80	2.00	1.30
13	0.03	6.5	25.0	8.20	0.00	1.60
14	0.03	6.5	32.0	4.00	0.00	0.20
15	0.03	6.5	28.5	6.00	0.00	1.40
16	0.03	6.5	28.5	6.80	0.00	1.50
17	0.03	6.5	28.5	5.80	0.00	1.40

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36 **Table S4.** model parameters of RS1, RS2, and RS3 response in the optimization test

Rs1	Coeff. SC	Std. Err.	P	Conf. int (±)
Constant	6.06	0.24	9.41E-12	0.52
A [mol/L]	1.93	0.20	5.66E-07	0.44
B	-1.61	0.20	3.79E-06	0.44
E [%]	-1.77	0.20	1.42E-06	0.44
B*B	-0.75	0.31	3.47E-02	0.68
N = 17	Q2 =	0.89	Cond. no. =	2.88
DF = 12	R2 =	0.95	RSD =	0.64
	R2 adj. =	0.94		
			Confidence =	0.95

Rs2~	Coeff. SC	Std. Err.	P	Conf. int (±)
Constant	0.45	0.07	1.31E-04	0.16
A [mol/l]	0.42	0.06	2.34E-05	0.13
B	-0.02	0.06	0.77	0.13
E [%]	-0.11	0.06	7.40E-02	0.13
B*B	0.60	0.10	1.72E-04	0.23
E*E	-0.16	0.10	0.15	0.23
A*B	0.11	0.06	0.12	0.14
N = 17	Q2 =	0.71	Cond. no. =	3.98
DF = 10	R2 =	0.91	RSD =	0.18
	R2 adj. =	0.85		
			Confidence =	0.95

Rs3~	Coeff. SC	Std. Err.	P	Conf. int (±)
Constant	1.32	0.002692	2.97E-23	6.00E-03
A [mol/l]	0.016	0.002253	2.97E-05	5.02E-03
B	0.0070	0.002253	1.14E-02	5.02E-03
E [%]	-0.0076	0.002253	7.37E-03	5.02E-03
B*B	-0.0075	0.003511	5.78E-02	7.82E-03

A*B	0.0047	0.002519	8.89E-02	5.61E-03
A*E	-0.0052	0.002519	6.36E-02	5.61E-03
N = 17	Q2 =	0.75	Cond. no. =	2.88
DF = 10	R2 =	0.90	RSD =	0.0071
	R2 adj. =	0.83		
			Confidence =	0.95

37 **Table S5.** Optimization criteria for Rs1, Rs2, and Rs3

Response	Criterion	Min	Target	Pred. min	Pred. max
Rs1	Maximize	1.20	4.00	-4.07E-06	10.62
Rs2	Maximize	1.20	3.00	-0.28	6.95
Rs3	Maximize	1.20	2.00	-0.22	2.80

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40 **Table S6.** Column parameters of chromatographic column

F	Column	H	S	A	B	C	C	Manufacturer
0	Hypersil GOLD	0.881	0.002	-0.017	0.036	0.162	0.479	Thermo/Hypersil
1.5	Acquity UPLC BEH Shield RP-18	0.907	0.016	-0.031	0.133	-0.055	0.416	Waters
1.92	Hypersil GOLD aQ	0.915	-0.010	-0.065	-0.019	0.371	0.638	Thermo/Hypersil
2.67	Capcell Pak C18 MGII	1.011	0.011	0.047	-0.006	0.007	-0.009	Shiseido
5.28	HSS T3	0.949	-0.021	-0.173	-0.002	0.031	0.180	Waters
5.49	Cortecs C18	1.075	0.043	-0.108	-0.037	0.063	0.035	Waters

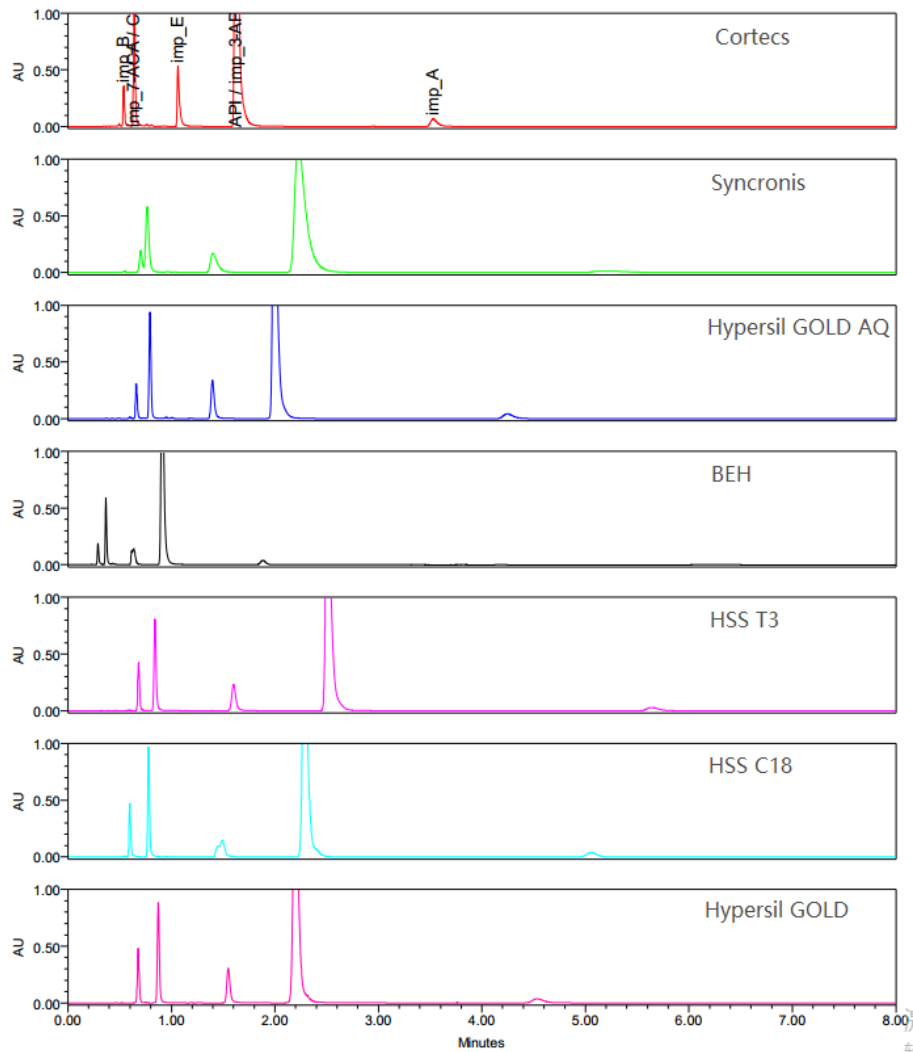
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45 **Figures**



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47 Fig. S1. Chromatogram of sample solution under different column conditions. The
 48 co-elution of 7-ACA and C is presented in the upper left second peak; the co-elution
 49 of API and 3-API is presented in the main peak.

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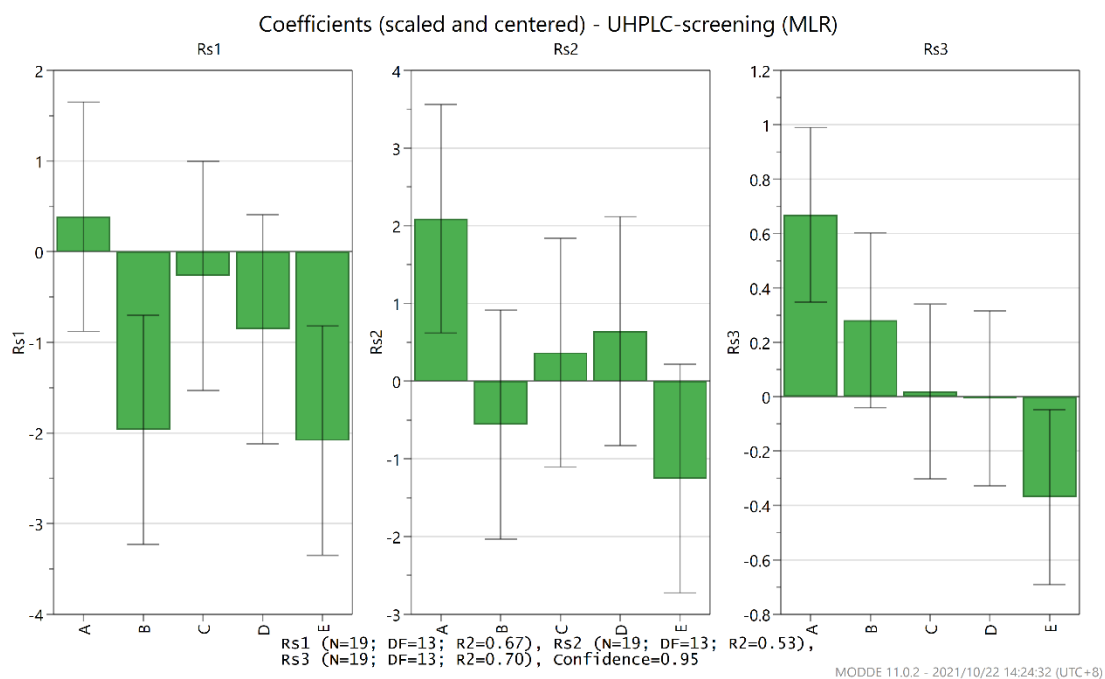
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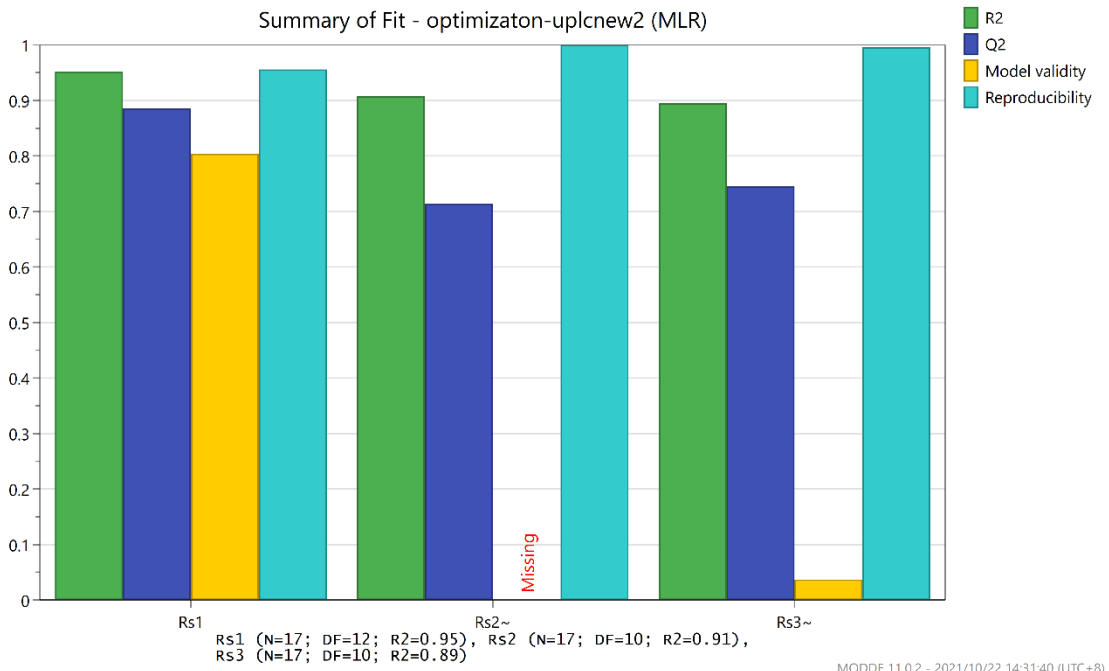
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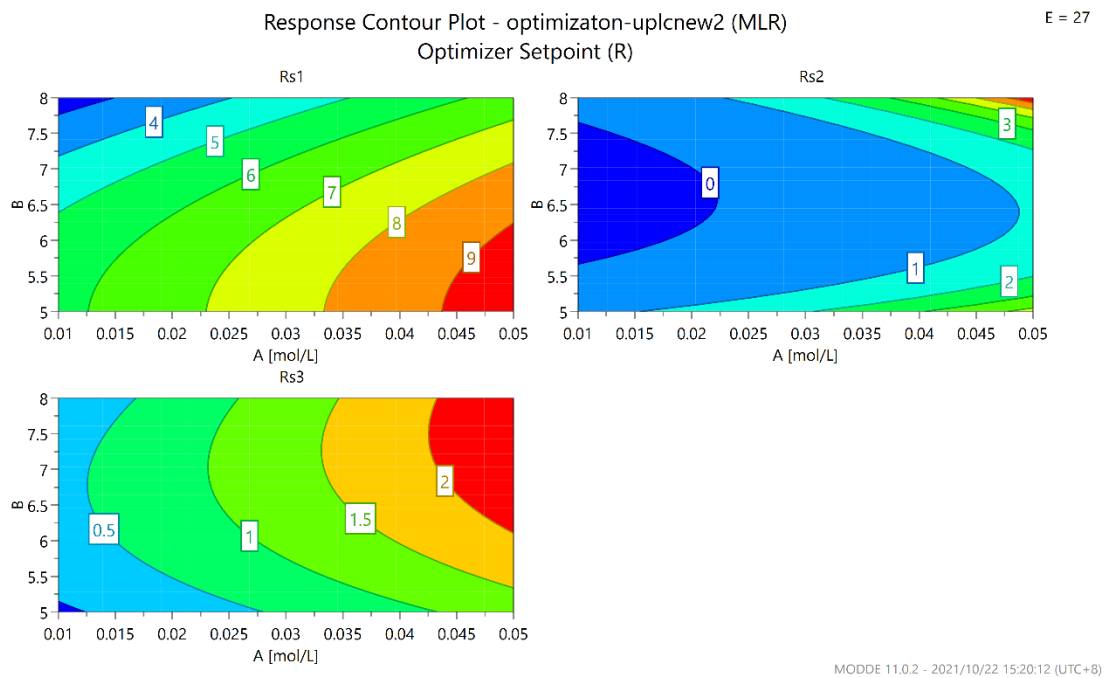
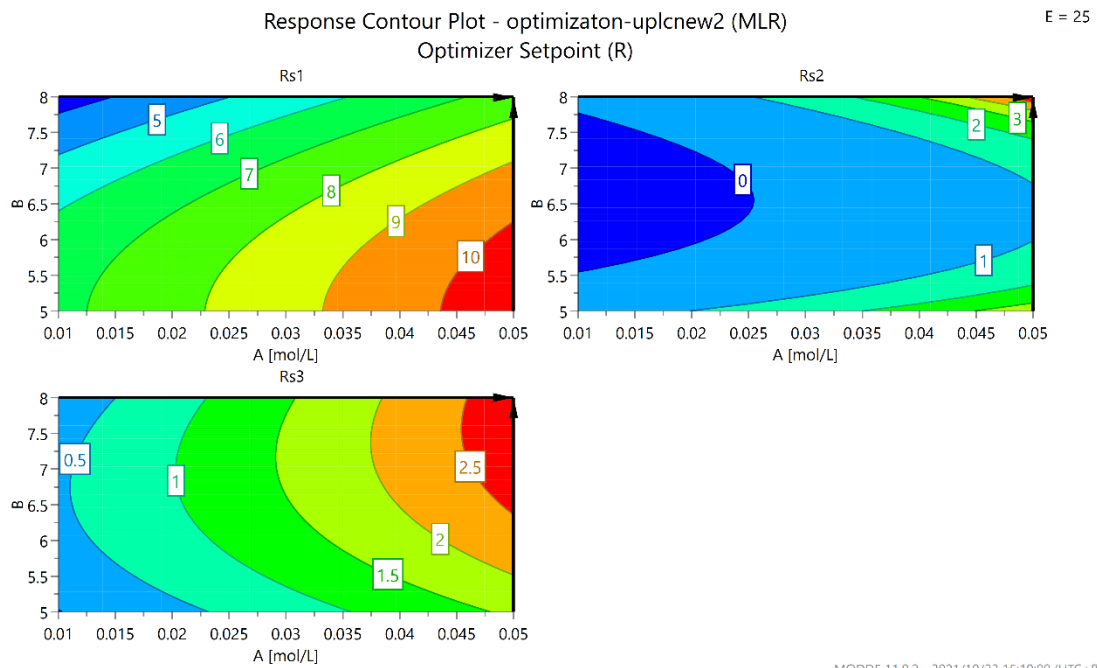
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57 **Fig. S2.** Coefficient plot for models created using screening design including
 58 coefficients and confidence interval. The effects of the five studied factors the
 59 concentration of octylamine (A, 0.01-0.05mM), pH value of mobile phase (B, 5-8),
 60 column temperature (C, 25-40°C), flow rate (D, 0.384-0.713mL / min), and organic
 61 phase ratio (E, 22-32%).and results of their significance tests for resolution between
 62 impurities B and 7-ACA (RS1), resolution between impurities 7-ACA and C (RS2), and
 63 resolution between impurities 3-API and ceftriaxone.

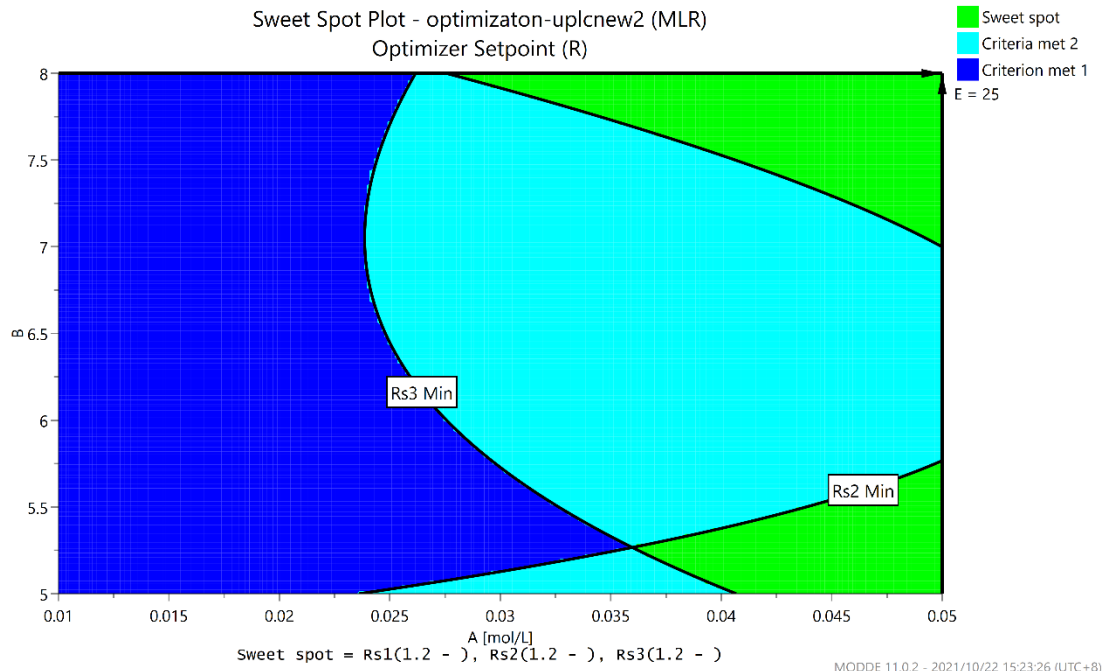


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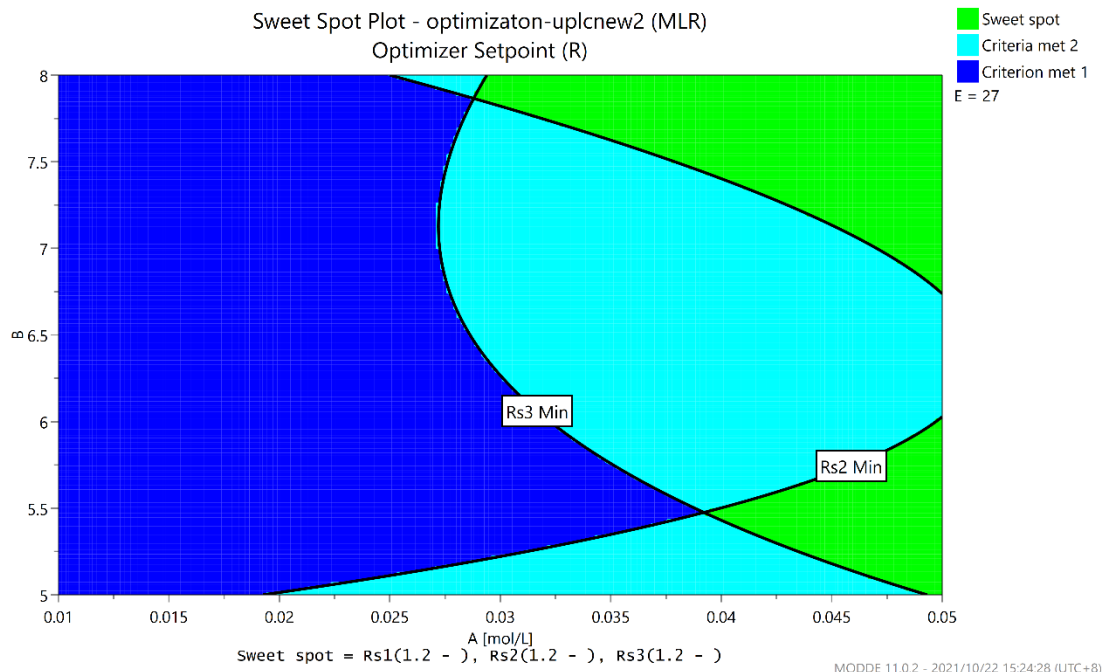
65 Fig. S3. Comparison diagram of model parameters of response results of
 66 optimization test (model validity for Rs2 is missing, because of the extremely good
 67 replicates).



71 Fig. S4. Isoresponse surfaces drawn for Rs1, Rs2, and Rs3 by plotting A vs. B at E
72 = 25% and 27%.



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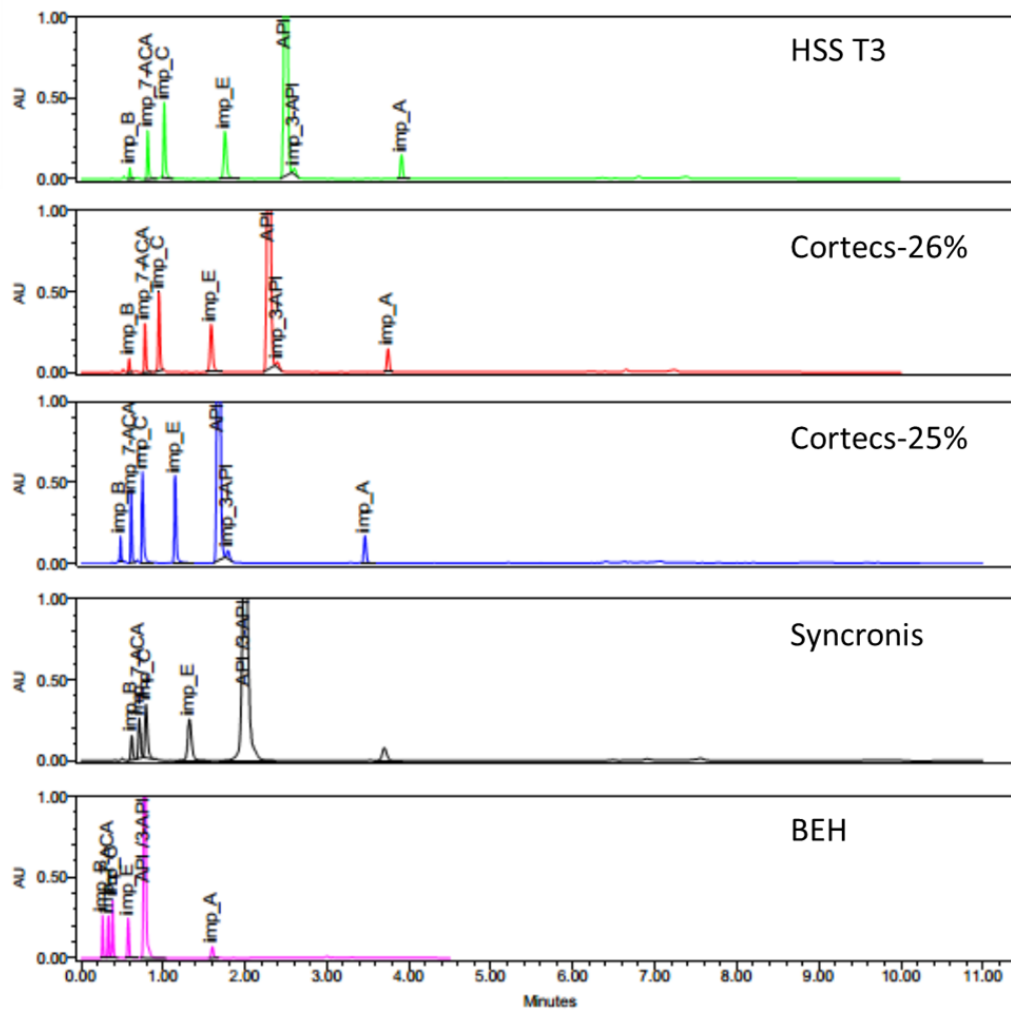


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75 Fig. S5. Sweet spot plots obtained by plotting A vs. B at (a) E = 25%; (b) E = 27%.

76 Green: all the requirements for the CMAs are fulfilled; pale blue: two requirements

77 fulfilled; dark blue: only one requirement fulfilled.



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79 **Fig. S6.** Typical chromatogram with different columns. Impurities B, 7-ACA, and C
 80 can be completely separated, however the resolution between impurity 3-API and
 81 ceftriaxone was affected by the chromatographic column. RS3 was 1.5 under the
 82 condition of 26% acetonitrile with Cortecs columns. After reducing the proportion of
 83 acetonitrile to 25%, RS3 increased to 1.8.