

Table S1: Recovery% for TMP,SDI, STZ, CTC, DOX, FF, NAL and FLU from spiked fish muscle by HPLC-UV method after using different extraction solvents .

Solvent	Compound recovery^a%							
	TMP	SDI	STZ	CTC	DOX	FF	NAL	FLU
Methanol	47.40	45.70	50.60	14.90	18.20	56.90	8.20	6.50
0.2%formic acid in methanol	63.00	87.00	92.40	43.80	40.90	95.80	14.30	9.80
0.2% formic acid in acetonitrile	55.80	85.20	91.00	57.00	44.00	83.00	92.90	95.20
0.2% formic acid in acetonitrile +Methanol	88.90	99.00	99.80	99.70	95.90	99.90	94.20	93.40

^a: Mean of three determinations.

Table S2: The system suitability test results of the developed HPLC-UV method for determination of TMP, SDI, STZ, CTC, DOX, FF, NAL and FLU .

Compound	Retention time (min)	Capacity factor (K')	Selectivity (α)^a	Resolution (R_s)^b	Tailing factor
TMP	4.58	4.39	1.23 _(a1)	3.82 _(b1)	0.96
SDI	5.44	5.41	1.16 _(a2)	2.87 _(b2)	0.81
STZ	6.22	6.32	1.75 _(a3)	17.56 _(b3)	0.79
CTC	10.27	11.09	1.09 _(a4)	5.49 _(b4)	1.10
DOX	11.19	12.16	1.16 _(a5)	9.90 _(b5)	0.99
FF	12.70	14.18	1.27 _(a6)	16.50 _(b6)	0.83
NAL	16.19	18.05	1.05 _(a7)	3.09 _(b7)	0.78
FLU	17.03	19.04			0.95

The retention time of unretained peak is 0.85 min.

a₁, b₁: are α and R_s calculated for SDI-TMP.

a₂, b₂: are α and R_s calculated for STZ-SDI.

a₃, b₃: are α and R_s calculated for CTC-STZ.

a₄, b₄: are α and R_s calculated for DOX-CTC.

a₅, b₅: are α and R_s calculated for FF-DOX.

a₆, b₆: are α and R_s calculated for NAL-FF.

a₇, b₇: are α and R_s calculated for FLU-NAL.

Table S3: Characteristic parameters of the calibration equations for the proposed HPLC-UV method for simultaneous determination of TMP, SDI, STZ, CTC, DOX, FF, NAL, and FLU in water.

Parameters	TMP	SDI	STZ	CTC	DOX	FF	NAL	FLU
Calibration range ($\mu\text{g ml}^{-1}$)	2-30	2-25	2-25	2-30	2-30	5-40	2-20	2-25
Detection limit ($\mu\text{g ml}^{-1}$)	0.016	0.024	0.019	0.014	0.02	0.025	0.005	0.01
Quantitation limit ($\mu\text{g ml}^{-1}$)	0.056	0.08	0.063	0.049	0.067	0.08	0.017	0.03
Regression equation (Y)^a: Slope (b)	37.41×10^3	13.18×10^4	11.20×10^4	47.81×10^3	41.80×10^3	61.56×10^2	23.33×10^4	11.82×10^4
Standard deviation of the slope (S_b)	274.69	1385.8	932.56	307.85	371.49	67.17	543.3	492.57
Relative standard deviation of the slope (%)	0.73	1.05	0.83	0.64	0.88	1.09	0.23	0.41
Confidence limit of the slope^b	$37.17 \times 10^3 - 37.64 \times 10^3$	$13.06 \times 10^3 - 13.30 \times 10^3$	$11.11 \times 10^4 - 11.28 \times 10^4$	$47.54 \times 10^3 - 48.08 \times 10^3$	$41.48 \times 10^3 - 42.12 \times 10^3$	$60.98 \times 10^2 - 62.14 \times 10^2$	$23.28 \times 10^4 - 23.38 \times 10^4$	$11.77 \times 10^4 - 11.86 \times 10^4$
Intercept (a)	109.2	1084.9	613.78	60.408	-120.39	-54.85	283.26	303.59
Standard deviation of the intercept (S_a)	481.89	1946.38	1309.74	540.05	651.71	169.61	604.1	691.79
Confidence limit of the intercept^b	$(-307.68) - 526.1$	$(-598.91) - 2768.76$	$(-519.29) - 1746.86$	$(-406.8) - 527.6$	$(-684.2) - 443.4$	$(-201.59) - 91.87$	$(-239.36) - 805.88$	$(-294.88) - 902.07$
Correlation coefficient (r)	0.9999	0.9999	0.9999	0.9998	0.9999	0.9999	0.9999	0.9999

^a $Y = a + bC$, where C is the concentration of compound in $\mu\text{g ml}^{-1}$ and Y is the peak area.

^b95 % confidence limit.

Table S4: Characteristic parameters of the calibration equations for the proposed HPLC-UV method for simultaneous determination of TMP, SDI, STZ, CTC, DOX, FF, NAL, and FLU in fish muscle.

Parameters	TMP	SDI	STZ	CTC	DOX	FF	NAL	FLU
Calibration range ($\mu\text{g kg}^{-1}$)	30-300	30-300	30-300	30-300	30-300	30-300	30-300	30-300
Detection limit ($\mu\text{g kg}^{-1}$)	0.03	0.02	0.04	0.03	0.03	0.03	0.02	0.04
Quantitation limit ($\mu\text{g kg}^{-1}$)	0.10	0.08	0.13	0.11	0.12	0.12	0.08	0.13
Regression equation (Y) ^a : Slope (b)	23.37	19.52	19.37	19.23	26.38	23.49	22.20	19.45
Standard deviation of the slope (S _b)	0.33	0.22	0.36	0.23	0.45	0.39	0.26	0.36
Relative standard deviation of the slope (%)	1.43	1.17	1.87	1.50	1.73	1.66	1.21	1.87
Confidence limit of the slope ^b	23.04–23.69	19.30–19.74	19.02–19.72	18.95–19.51	25.94–26.83	23.11–23.87	21.94–22.46	19.09–19.80
Intercept (a)	504.59	989.72	1142.90	788.11	384.89	273.42	2098.95	2091.34
Standard deviation of the intercept (S _a)	60.40	41.30	65.54	52.16	82.87	70.74	48.76	65.86
Confidence limit of the intercept ^b	445.91 – 563.28	949.60 – 1029.85	1079.22 – 1206.58	737.42 – 838.79	304.37 – 465.41	204.68 – 342.16	2051.57 – 2146.33	2027.35 – 2155.33
Correlation coefficient (r)	0.9998	0.9998	0.9997	0.9998	0.9997	0.9997	0.9998	0.9997

^a $Y = a + bC$, where C is the concentration of compound in $\mu\text{g kg}^{-1}$ and Y is the peak area.

^b95 % confidence limit.

Table S5: Characteristic parameters of the calibration equations for LC-MS method for simultaneous determination of SDI, CTC and FF in water sample.

Parameters	SDI	CTC	FF
Calibration range (ng ml⁻¹)	0.001-100	0.001-60	0.001-20
Detection limit (ng ml⁻¹)	0.02	0.03	0.04
Quantitation limit (ng ml⁻¹)	0.07	0.12	0.14
Regression equation (Y)^a:	21.64	16.01	5.27
Slope (b)			
Standard deviation of the slope (S_b)	0.23	0.27	0.10
Relative standard deviation of the slope (%)	1.08	1.69	2.02
Confidence limit of the slope ^b	21.41 –21.87	15.75 -16.28	5.16–5.37
Intercept (a)	-14.89	6.58	1.75
Standard deviation of the intercept (S_a)	36.09	9.77	0.11
Confidence limit of the intercept ^b	-49.95–20.17	-2.92-16.08	1.64–1.85
Correlation coefficient (r)	0.9999	0.9998	0.9997
Standard error of estimation	23.36	5.42	0.0776

^a $Y = a + bC$, where C is the concentration of compound in ng ml⁻¹ and Y is the peak area.

^b95 % confidence limit.

Table S6: Characteristic parameters of the calibration equations for LC-MS method for simultaneous determination of SDI, CTC and FF in fish muscle.

Parameters	SDI	CTC	FF
Calibration range ($\mu\text{g kg}^{-1}$)	10-150	10-150	10-150
Detection limit ($\mu\text{g kg}^{-1}$)	0.03	0.04	0.04
Quantitation limit ($\mu\text{g kg}^{-1}$)	0.12	0.14	0.13
Regression equation (Y)^a: Slope (b)	42.89	315.30	178.76
Standard deviation of the slope (S_b)	0.74	6.07	3.39
Relative standard deviation of the slope (%)	1.74	1.92	1.89
Confidence limit of the slope ^b	42.17 –43.62	309.96 – 321.20	172.46–182.05
Intercept (a)	-16.53	9.59	11.94
Standard deviation of the intercept (S_a)	58.55	475.02	14.89
Confidence limit of the intercept ^b	-73.42 – 40.35	-451.96 – 471.09	-2.52–26.41
Correlation coefficient (r)	0.9997	0.9997	0.9997

^a $Y = a + bC$, where C is the concentration of compound in $\mu\text{g kg}^{-1}$ and Y is the peak area.

^b95 % confidence limit.

Table S7: a) Factors for robustness study and b) responses measured.

a)Factors for robustness study.

Independent factors	Levels		
	Level(-1)	Level(0)	Level(+1)
A- ACN% start	8	10	12
B- ACN% end	38	40	42
C-Column temperature (°C)	23	25	27
D-flow rate(ml/min)	1.1	1.2	1.3

b)responses measured.

Std	Run	A	B	C	D	Responses			
						Rt(TMP)	Rt(FLU)	Rs(SDI-STZ)	Rs(NAL-FLU)
5	1	8	38	27	1.3	4.70	18.80	2.88	4.06
1	2	8	38	23	1.1	6.70	16.80	1.96	3.66
2	3	12	38	23	1.3	4.14	15.75	2.29	3.51
9	4	10	40	25	1.2	4.58	17.03	2.80	3.09
8	5	12	42	27	1.3	4.20	17.80	2.80	3.70
7	6	8	42	27	1.1	6.40	17.39	2.88	3.17
4	7	12	42	23	1.1	4.70	18.08	2.12	4.05
3	8	8	42	23	1.3	5.10	17.05	2.15	3.81
6	9	12	38	27	1.1	4.15	16.76	1.57	4.14

Table S8: ANOVA results for robustness test. A 5% level of significance was desired.

Factors	Rt(TMP)		Rt(FLU)		Rs(SDI-STZ)		Rs(NAL-FLU)	
	F	p*	F	p*	F	p*	F	p*
A-ACN% start	13.85816	0.0204	0.336647	0.5929	0.715766	0.4452	0.109375	0.6519
B-ACN% end	0.214264	0.6675	0.603937	0.4805	0.941322	0.3869	0.236931	0.6793
C-Temperature	0.601904	0.4812	1.165424	0.3411	1.5616	0.2796	0.198055	0.9791
D-Flow rate	6.169975	0.0679	0.016928	0.9028	1.523044	0.2847	0.000774	0.9687

* p-value should be less than 0.05 to be statistically significant

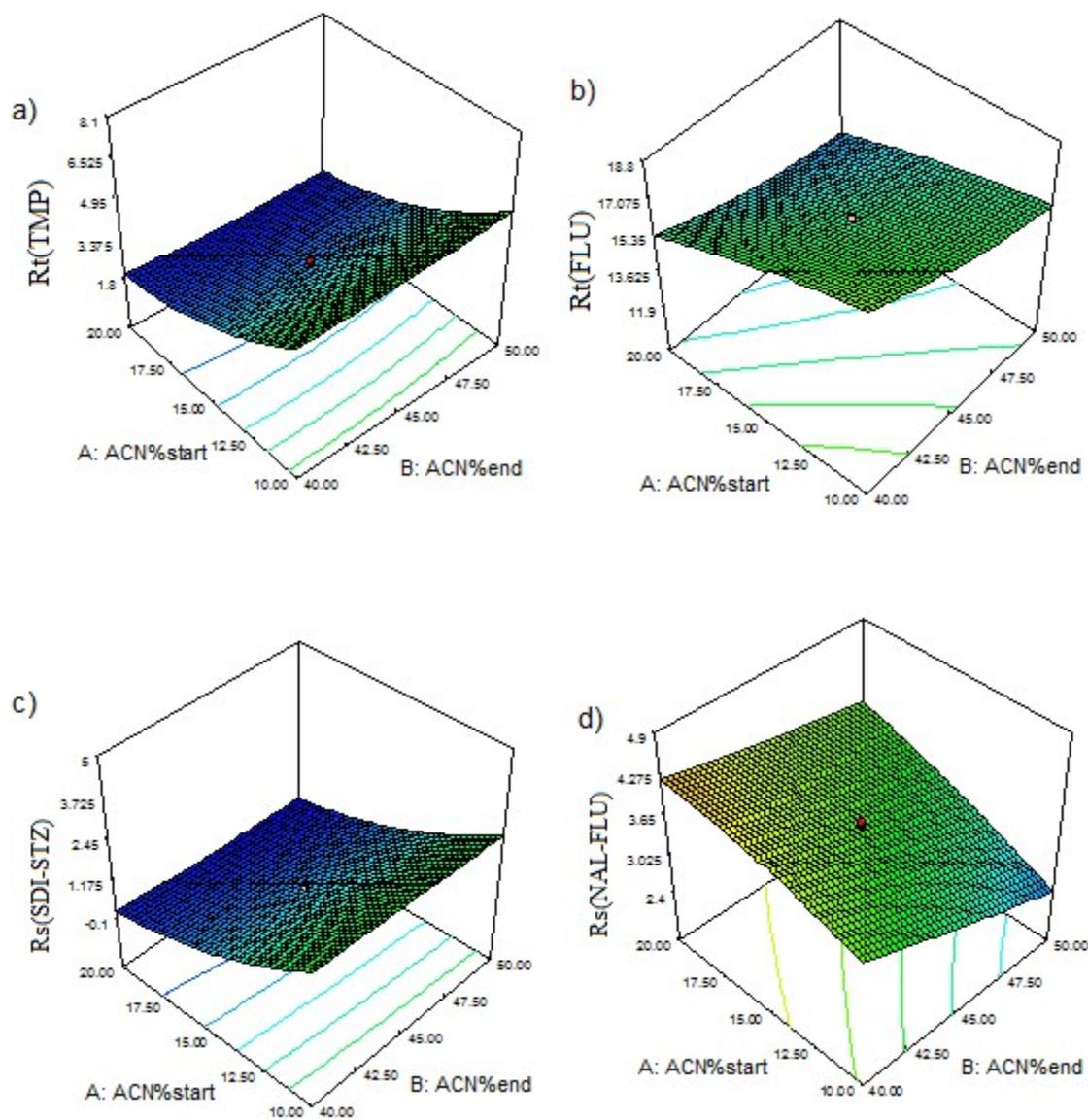


Fig. S1: Responses surfaces related to the interaction effects of the initial fraction of mobile phase B(ACN% start) and the final fraction of mobile phase B (ACN% end): a) the retention time of the first eluted peak R_t (TMP) ,b) the retention time of the last eluted peak R_t (FLU) ,c) the resolution of critical pair R_s (SDI-STZ) and d) the resolution of critical pair R_s (NAL-FLU) .Column temperature was kept constant at 25°C and flow rate was 1 ml/min.

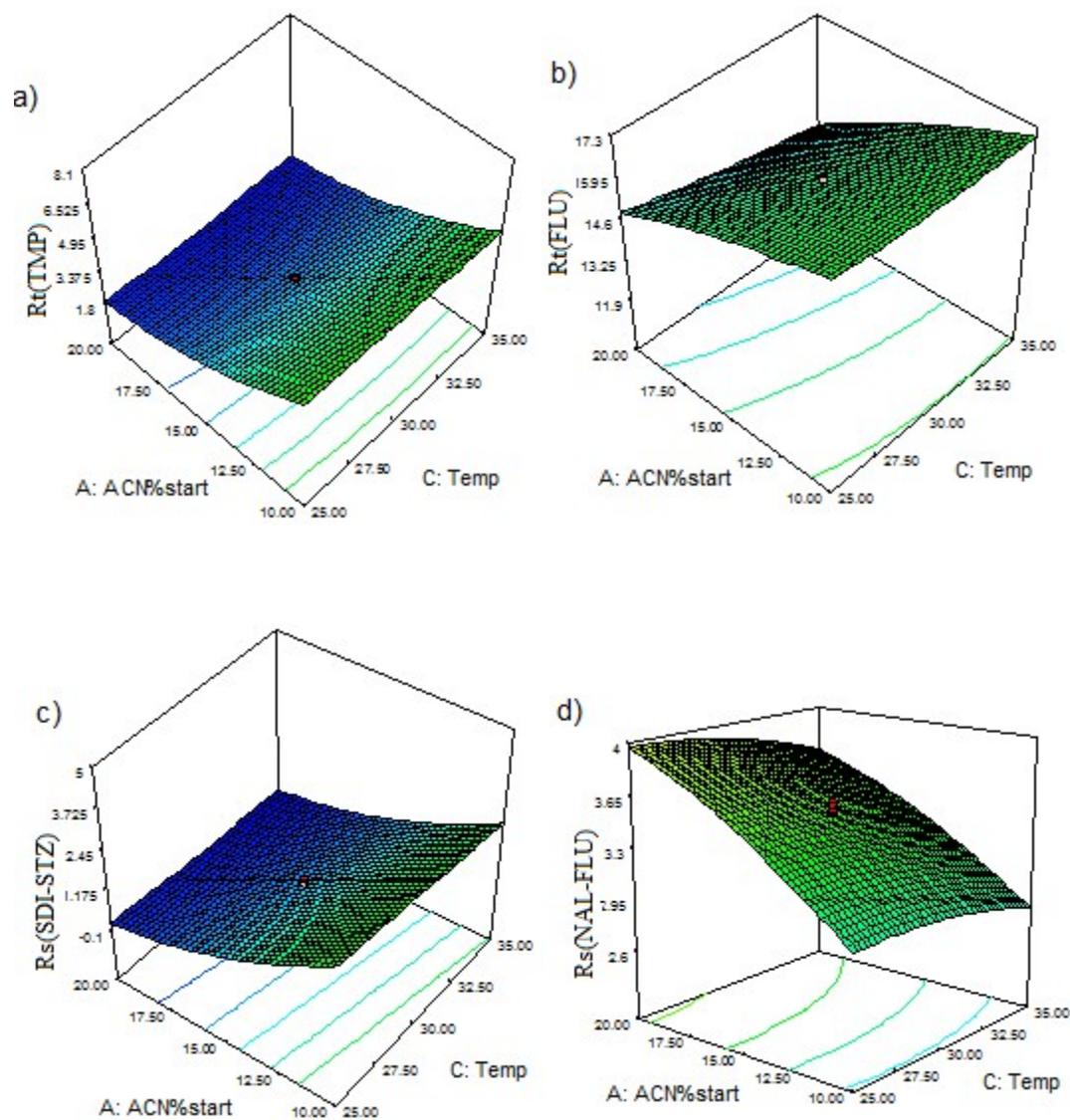


Fig. S2: Responses surfaces related to the interaction effects of the initial fraction of mobile phase B(ACN% start) and Column temperature: a) the retention time of the first eluted peak $R_t(\text{TMP})$,b) the retention time of the last eluted peak $R_t(\text{FLU})$, c) the resolution of critical pair $R_s(\text{SDI-STZ})$ and d) the resolution of critical pair $R_s(\text{FLU-NAL})$. the final fraction of mobile phase B (ACN% end) was kept constant at 45% and flow rate of the mobile phase was 1 ml/min.

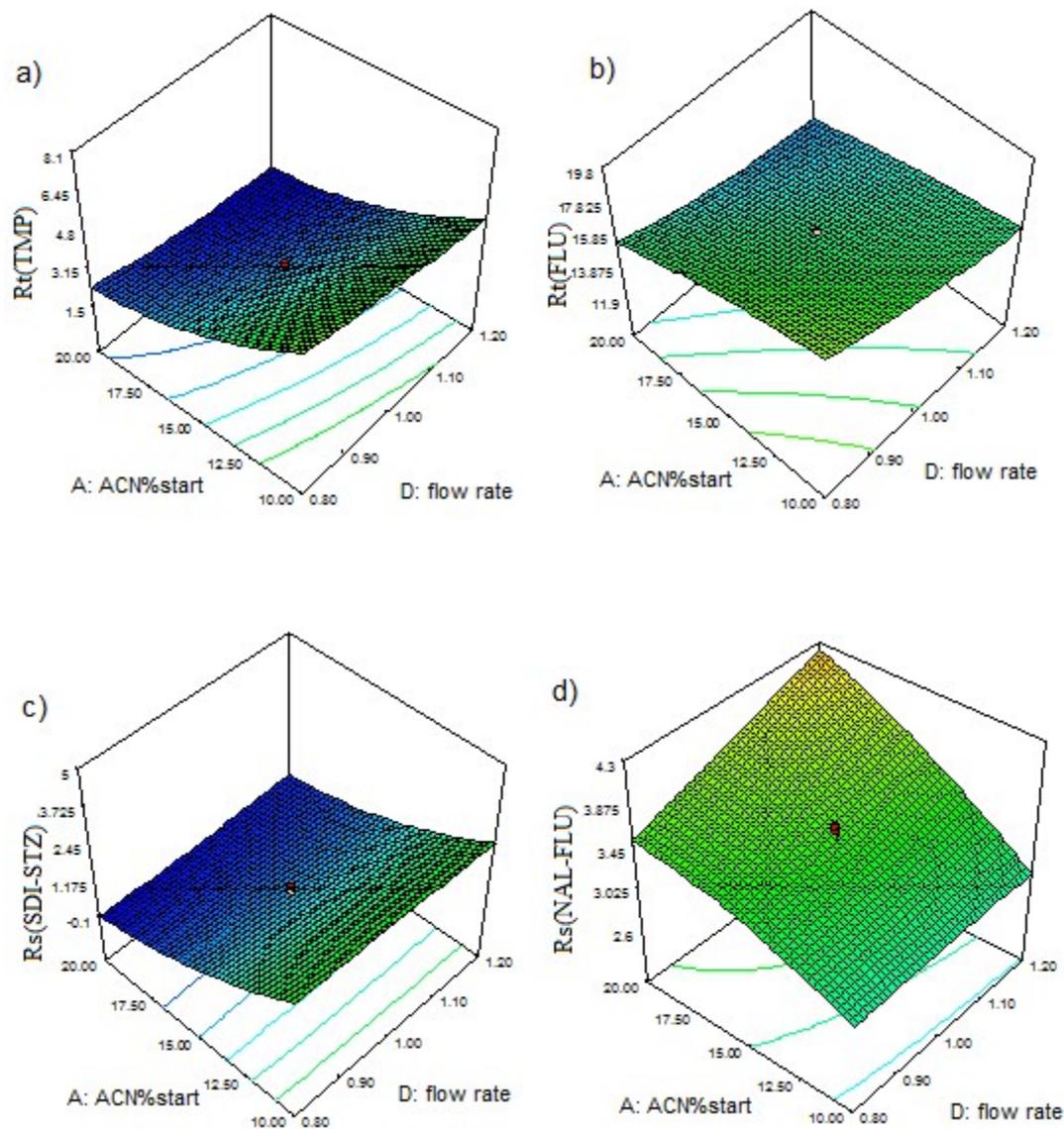


Fig. S3: Responses surfaces related to the interaction effects of the initial fraction of mobile phase B(ACN% start) and flow rate of the mobile phase: a) the retention time of the first eluted peak $R_t(\text{TMP})$, b) the retention time of the last eluted peak $R_t(\text{FLU})$, c) the resolution of critical pair $R_s(\text{SDI-STZ})$ and d) the resolution of critical pair $R_s(\text{FLU-NAL})$. Column temperature was kept constant at 30°C and the final fraction of mobile phase B (ACN% end) was at 45%.

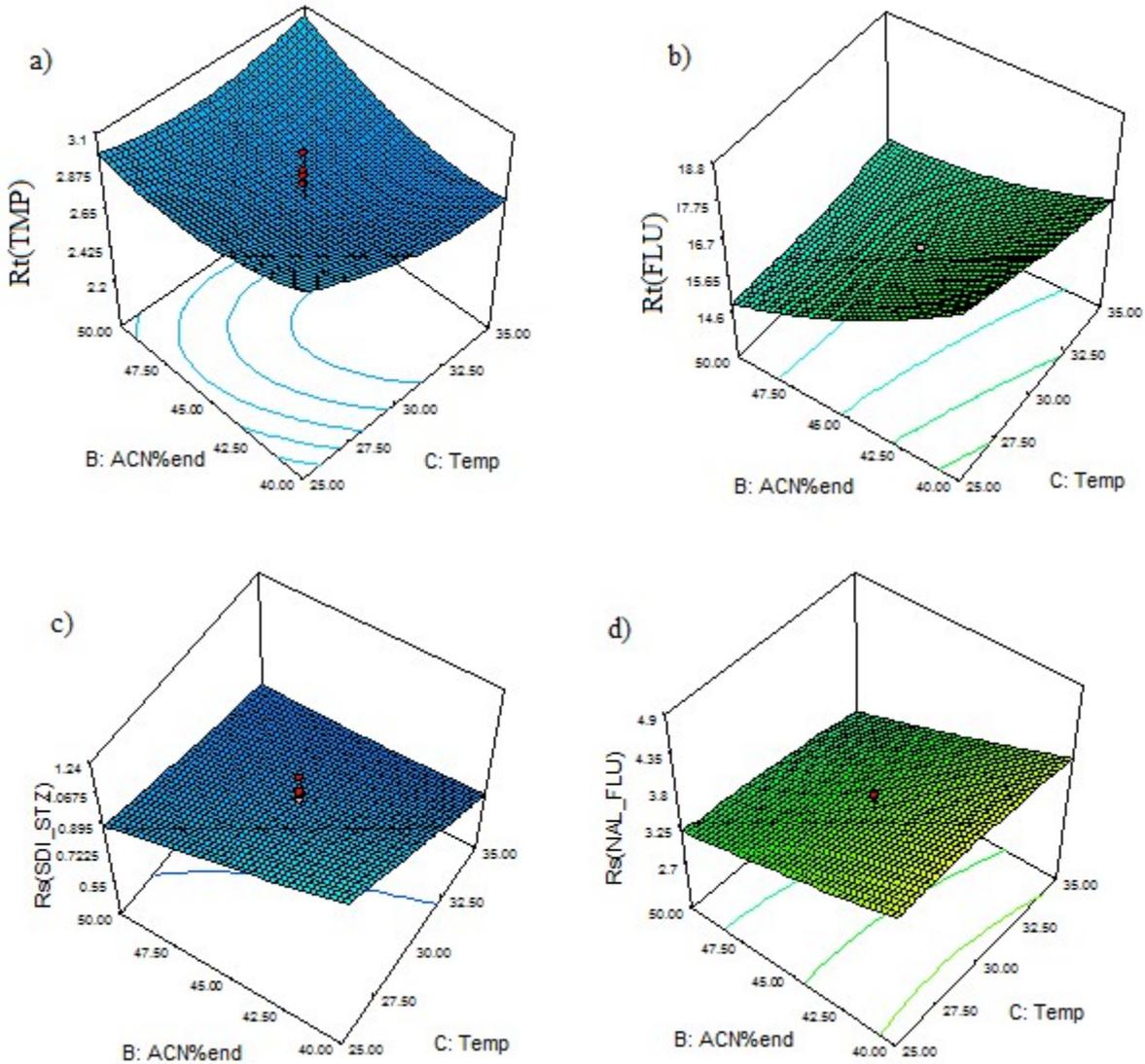


Fig. S4: Responses surfaces related to the interaction effects of the final fraction of mobile phase B (ACN% end) and Column temperature: a) the retention time of the first eluted peak $Rt(TMP)$,b) the retention time of the last eluted peak $Rt(FLU)$,c) the resolution of critical pair $Rs(SDI-STZ)$ and d) the resolution of critical pair $Rs(NAL-FLU)$. the initial fraction of mobile phase B(ACN% start) was kept constant at 15% and flow rate of the mobile phase was 1 ml/min.

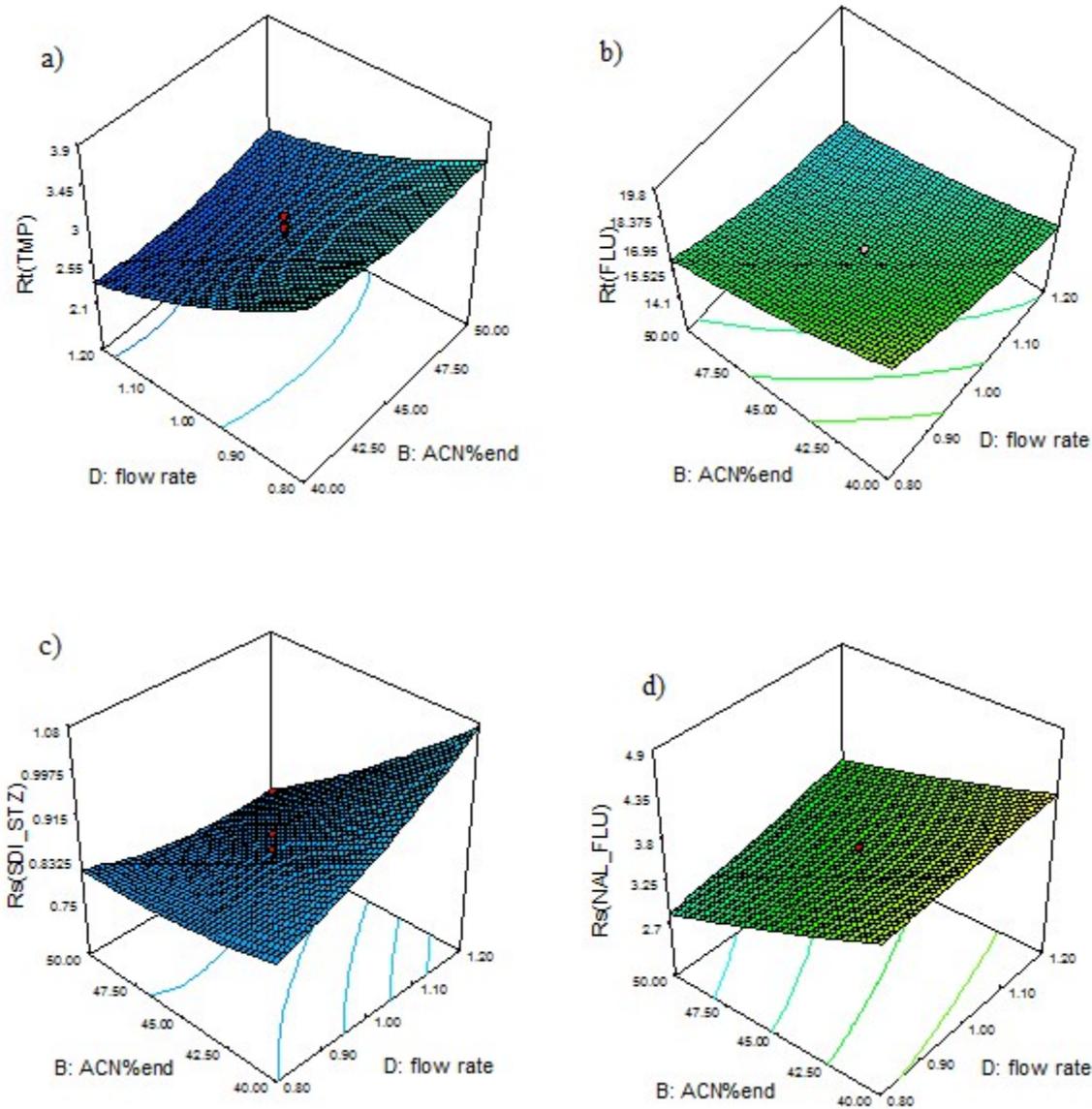


Fig. S5: Responses surfaces related to the interaction effects of the final fraction of mobile phase B (ACN% end) and flow rate of the mobile phase: a) the retention time of the first eluted peak $Rt(TMP)$, b) the retention time of the last eluted peak $Rt(FLU)$, c) the resolution of critical pair $Rs(SDI-STZ)$ and d) the resolution of critical pair $Rs(FLU-NAL)$. Column temperature was kept constant at 30°C and the initial fraction of mobile phase B (ACN% start) was 15%.

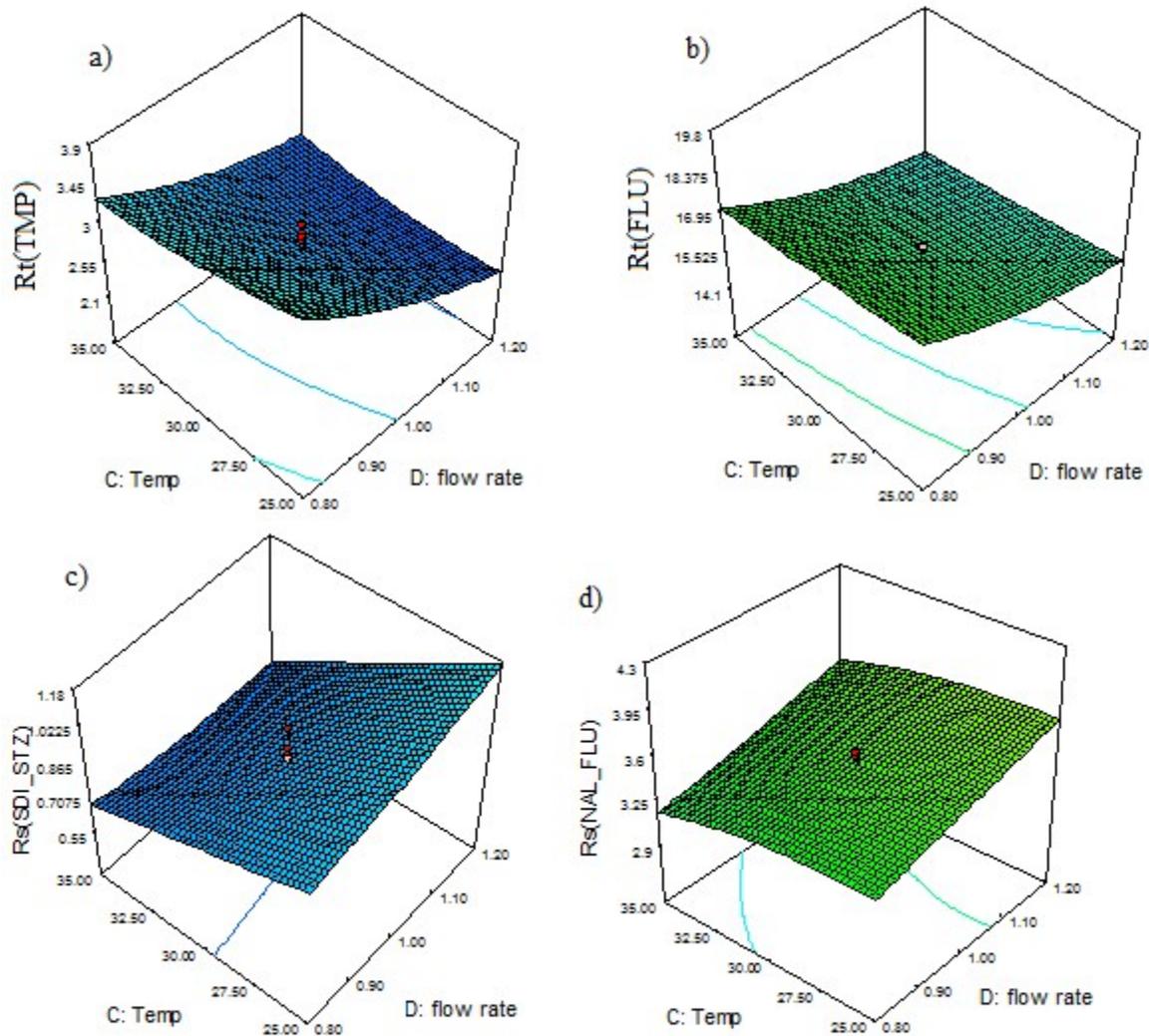


Fig. S6: Responses surfaces related to the interaction effects of Column temperature and flow rate of the mobile phase : a) the retention time of the first eluted peak $R_t(\text{TMP})$,b) the retention time of the last eluted peak $R_t(\text{FLU})$, c) the resolution of critical pair R_s (SDI-STZ) and d) the resolution of critical pair R_s (FLU-NAL) . The final fraction of mobile phase B (ACN% end) was kept constant at 45%. and the initial fraction of mobile phase B(ACN% start) was 15%.

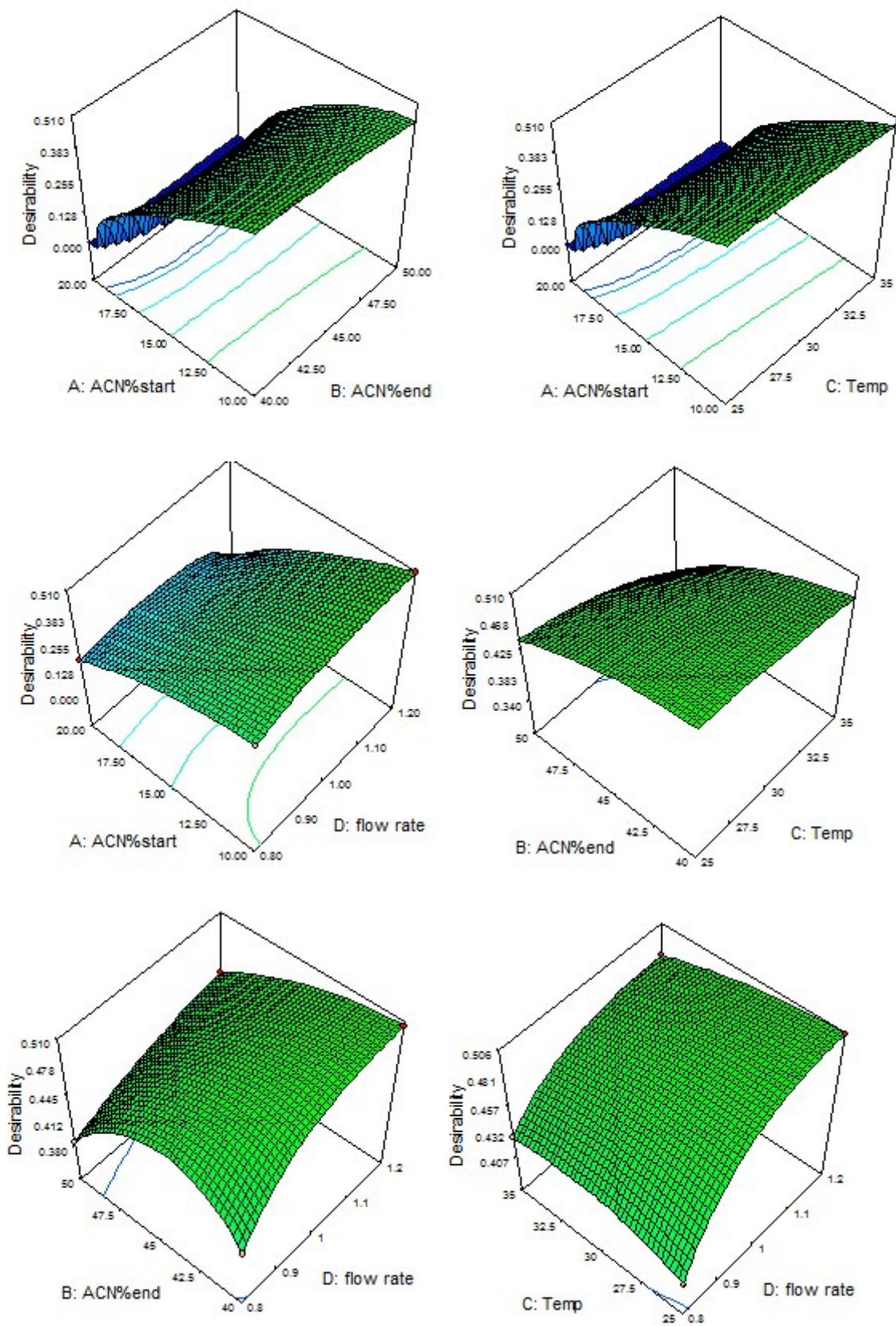


Fig. S7: Response surface obtained for desirability function for different two factors interaction.