

Supplementary material

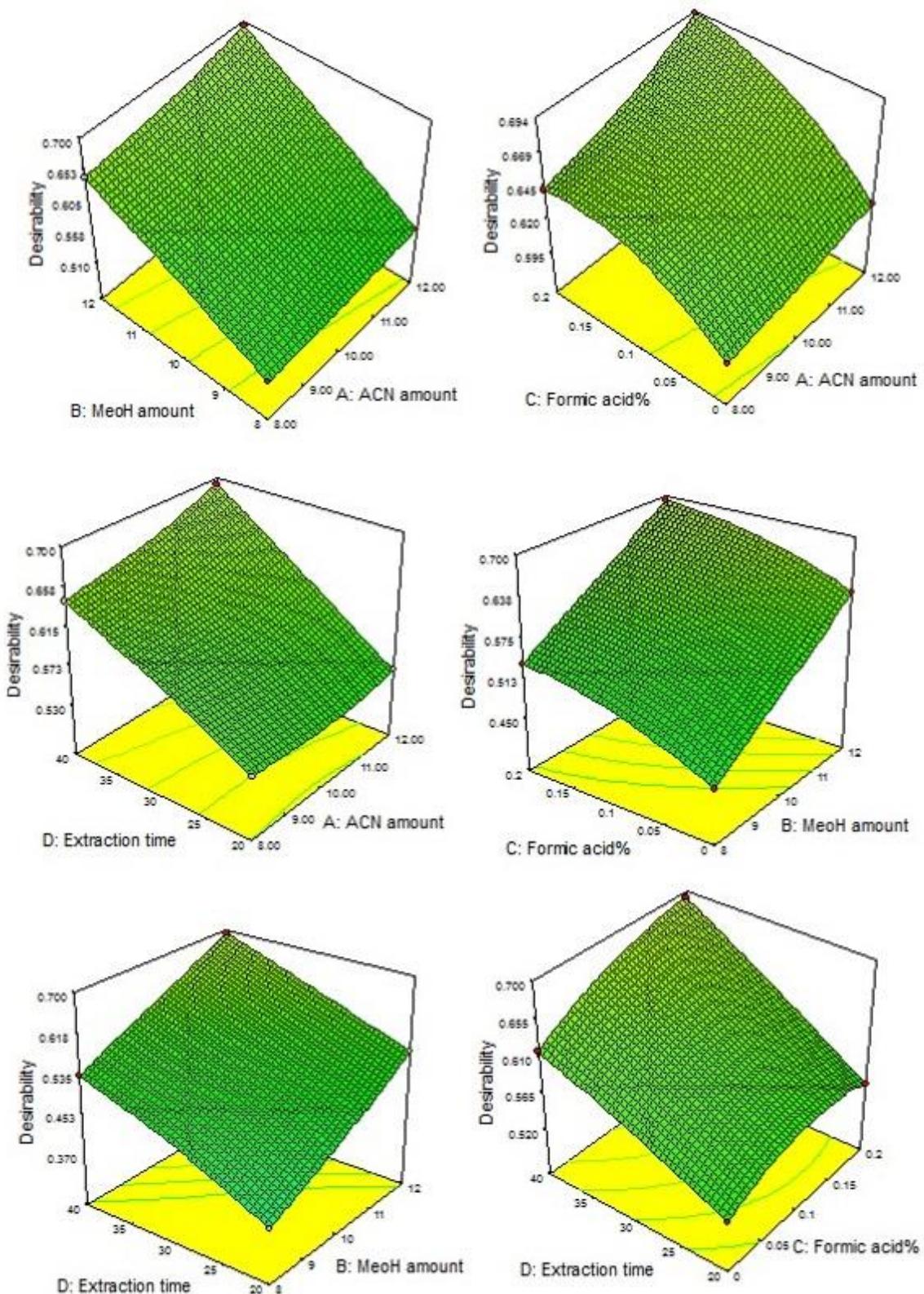


Figure S1. Response surface obtained for desirability function for different two factors interaction for optimization of extraction procedure.

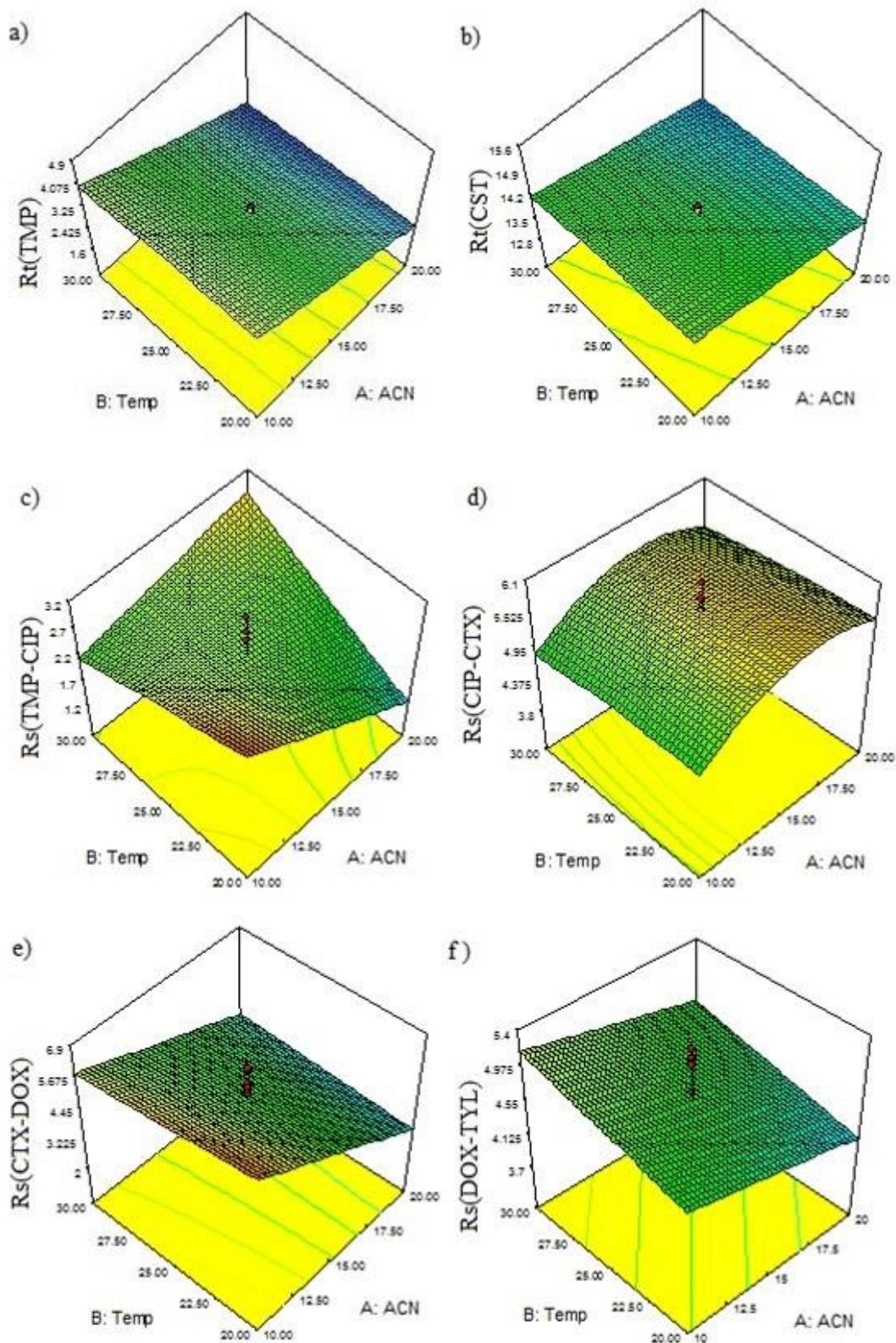


Figure S2. Responses surfaces related to the interaction effects of the percentage of acetonitrile and Column temperature: a) retention time of the first peak (TMP), b) retention time of the last peak (CST), c) resolution (TMP-CIP) peaks, d) resolution of (CIP-CTX), e) resolution (CTX-DOX) peaks and f) resolution (DOX-TYL) peaks. The flow rate of mobile phase was kept constant at 1 ml min^{-1} .

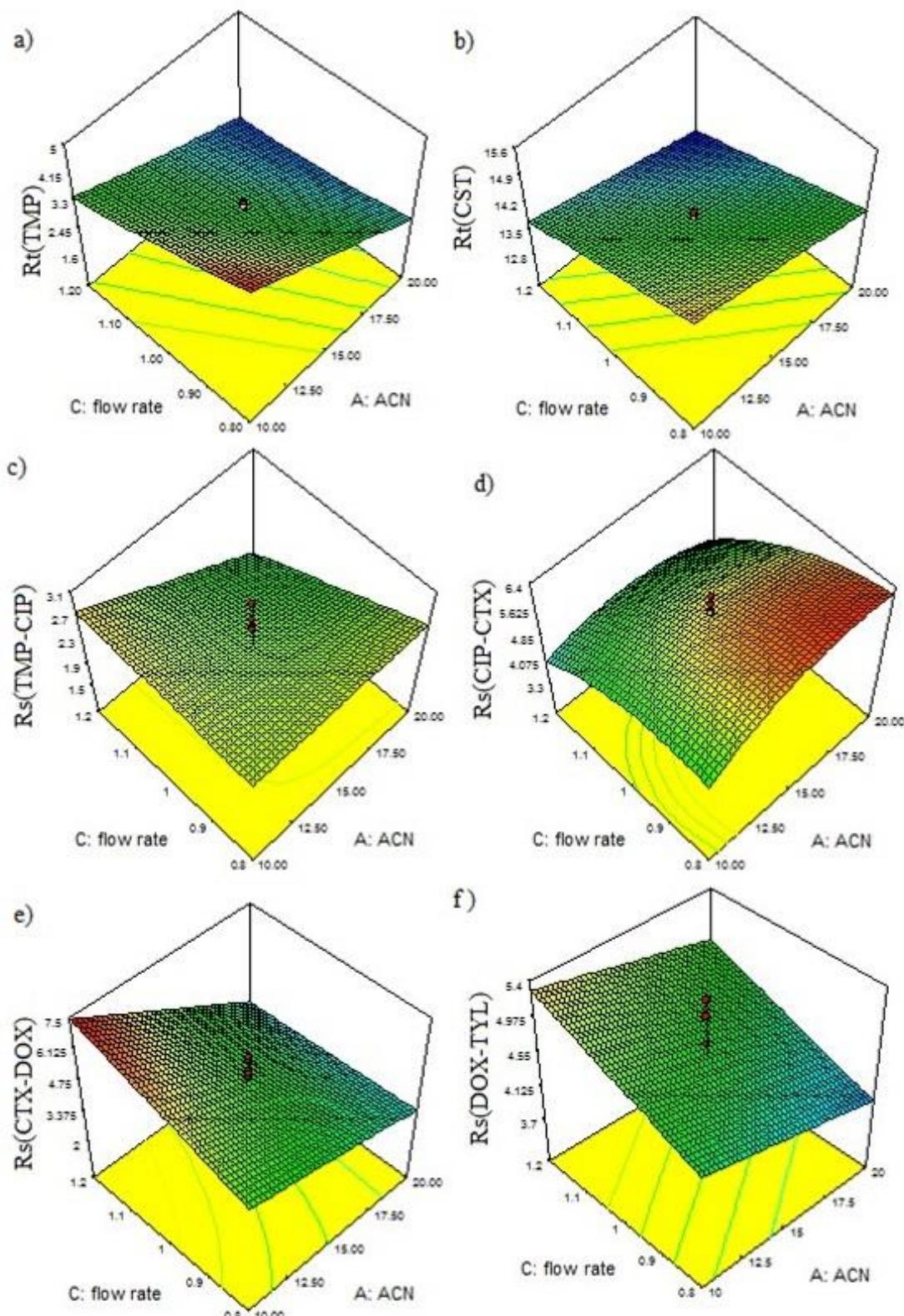


Figure S3. Responses surfaces related to the interaction effects of the percentage of acetonitrile and flow rate of the mobile phase: a) retention time of the first peak (TMP), b) retention time of the last peak (CST), c) resolution of (TMP-CIP) peaks, d) resolution of (CIP-CTX) peaks, e) resolution of peaks (CTX-DOX) peaks and f) resolution of (DOX-TYL) peaks. The column temperature was kept constant at 30 °C.

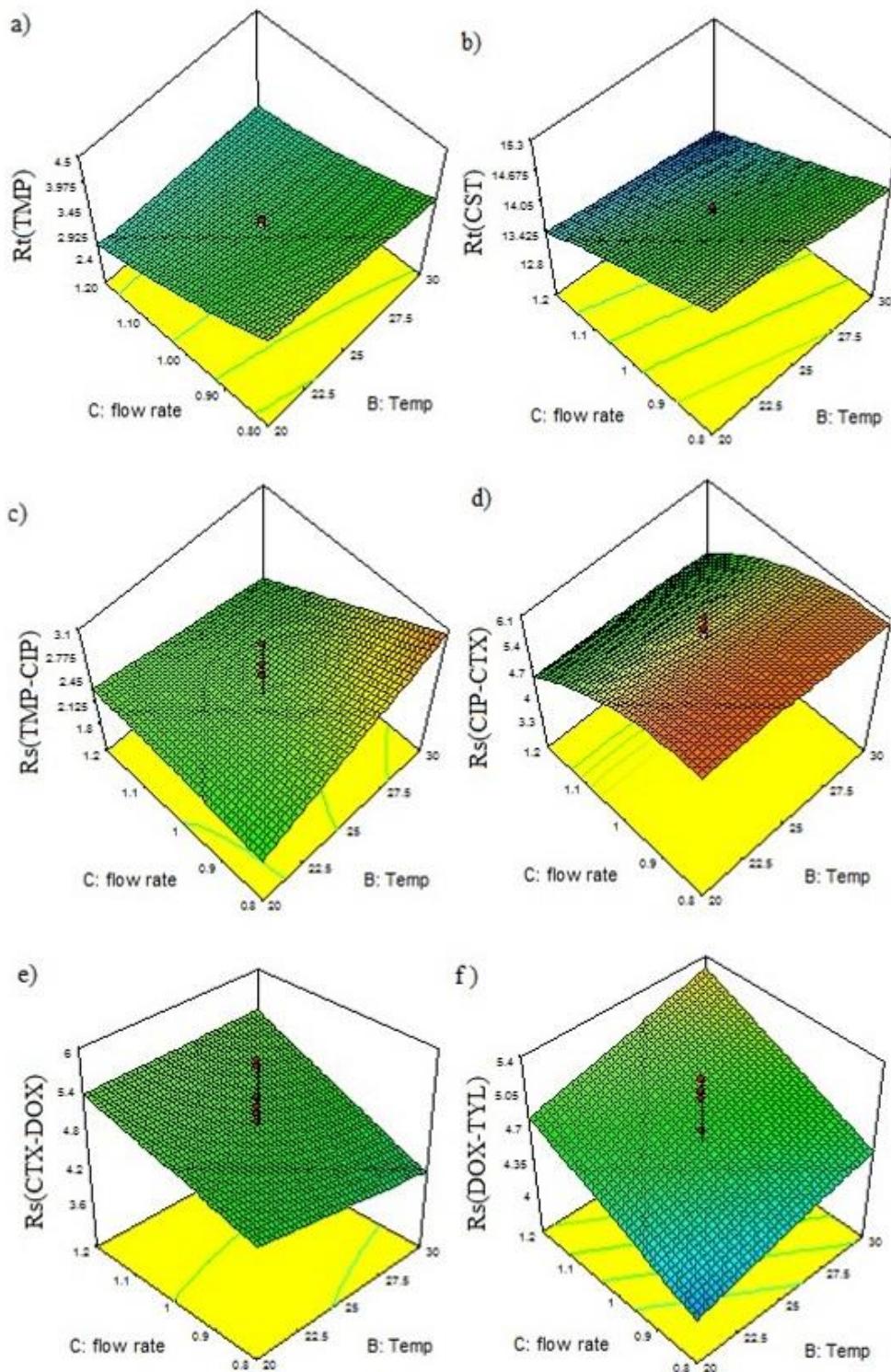


Figure S4. Responses surfaces related to the interaction effects of Column temperature and flow rate of the mobile phase: a) retention time of the first peak (TMP), b) retention time of the last peak (CST), c) resolution of (TMP-CIP) peaks, d) resolution of (CIP-CTX) peaks, e) resolution of (CTX-DOX) peaks and f) resolution (DOX-TYL) peaks. the percentage of acetonitrile was kept constant at 10%.

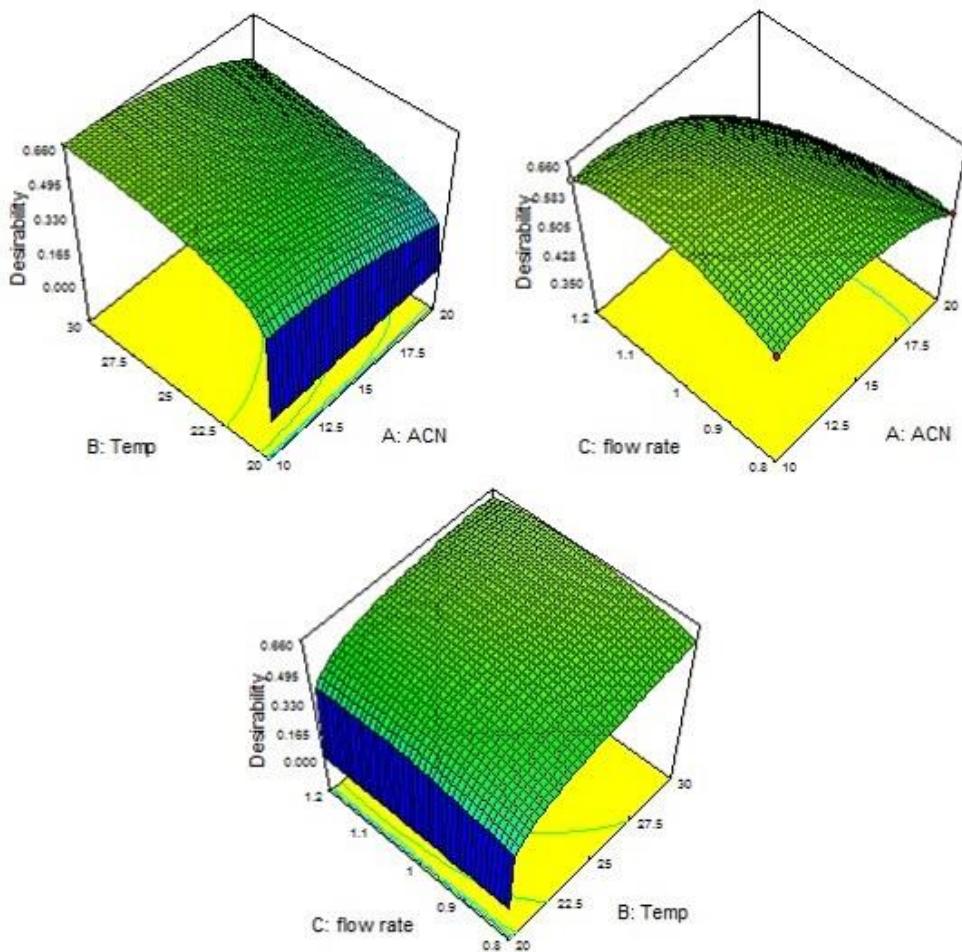


Figure S5. Response surface obtained for desirability function for different two factors interaction for optimization of HPLC-UV method.

Table S1. (a) Factors examined in the screening phase for factors affecting extraction procedure (Fractional factorial design)., (b) Matrix of the design. (c) The corresponding experimental response values for fractional factorial design.

Table S1. (a)

Independent factors	Levels		
	Level (-1)	Level (0)	Level (+1)
A- Methanol amount (mL)	8	10	12
B- Acetonitrile amount (mL)	8	10	12
C-Formic acid %	0	0.1	0.2
D-Extraction time (min)	20	30	40

Table S1. (b)

Std orde r	Run order	Experimental design				Experimental set-up			
		Factor 1	Factor 2	Factor 3	Factor 4	MeOH amount (mL)	ACN amount (mL)	Formic acid% %	Extraction Time (min)
6	1	1	-1	1	-1	12	8	0.2	20
2	2	1	-1	-1	1	12	8	0	40
1	3	-1	-1	-1	-1	8	8	0	20
7	4	-1	1	1	-1	8	12	0.2	20
3	5	-1	1	-1	1	8	12	0	40
8	6	1	1	1	1	12	12	0.2	40
5	7	-1	-1	1	1	8	8	0.2	40
4	8	1	1	-1	-1	12	12	0	20
9	9	0	0	0	0	10	10	0.1	30

Table S1. (c)

Run order	Responses							
	R%(TMP)	R%(CIP)	R%(CTX)	R%(DOX)	R%(TYL)	R%(SMZ)	R%(FLU)	R%(CST)
1	66.86	64.72	88.2	104.61	83.52	97.24	94.77	84.41
2	73.17	89	57.23	93.6	20.33	83.73	76.83	75.81
3	89.52	88.83	97.45	76.12	74.99	90.19	90.06	92.14
4	59.23	98.11	99.45	106.98	83.09	110.29	91.72	99.53
5	85.96	93.44	57.48	97.98	32.22	88.65	77.94	79.42
6	55.57	87.41	75.2	105.64	79.21	101.2	106.31	72.35
7	90.73	110.21	92.8	88.05	97.91	94.76	90.89	90.81
8	56.26	92.57	81.49	89.97	70.75	89.67	102.87	77.94
9	65.78	79.76	94.89	93.96	77.49	100.92	101.49	88.11

Table S2. CCD for optimization of the factors affecting the extraction procedure. (a) Matrix of the design, (b) the corresponding experimental response values.

Table S2 (a)

Std order	Run order	Experimental design				Experimental set-up			
		Factor 1	Factor 2	Factor 3	Factor 4	MeoH amount (mL)	ACN amount (mL)	Formic acid%	Extraction Time (min)
1	1	-1	-1	-1	-1	8	8	0	20
25	2	0	0	0	0	10	10	0.1	30
22	3	0	0	2	0	10	10	0.3	30
21	4	0	0	-2	0	10	10	-0.1	30
2	5	1	-1	-1	-1	12	8	0	20
26	6	0	0	0	0	10	10	0.1	30
15	7	-1	1	1	1	8	12	0.2	40
16	8	1	1	1	1	12	12	0.2	40
29	9	0	0	0	0	10	10	0.1	30
13	10	-1	-1	1	1	8	8	0.2	40
17	11	-2	0	0	0	6	10	0.1	30
14	12	1	-1	1	1	12	8	0.2	40
5	13	-1	-1	1	-1	8	8	0.2	20
11	14	-1	1	-1	1	8	12	0	40
28	15	0	0	0	0	10	10	0.1	30
30	16	0	0	0	0	10	10	0.1	30
9	17	-1	-1	-1	1	8	8	0	40
23	18	0	0	0	-2	10	10	0.1	10
19	19	0	-2	0	0	10	6	0.1	30
8	20	1	1	1	-1	12	12	0.2	20
24	21	0	0	0	2	10	10	0.1	50
12	22	1	1	-1	1	12	12	0	40
18	23	2	0	0	0	14	10	0.1	30
6	24	1	-1	1	-1	12	8	0.2	20
20	25	0	2	0	0	10	14	0.1	30
27	26	0	0	0	0	10	10	0.1	30
4	27	1	1	-1	-1	12	12	0	20
7	28	-1	1	1	-1	8	12	0.2	20
10	29	1	-1	-1	1	12	8	0	40
3	30	-1	1	-1	-1	8	12	0	20

Table S2 (b)

Run order	Responses							
	R%(TMP)	R%(CIP)	R%(CTX)	R%(DOX)	R%(TYL)	R%(SMZ)	R%(FLU)	R%(CST)
1	79.52	88.88	77.86	76.12	74.99	91.02	89.99	91.89
2	87.97	78.78	88.99	93.96	77.49	99.89	102.29	89.77
3	63.95	82.09	89.31	71.66	88.65	94.56	75.28	94.12
4	78.71	73.88	88.79	84	68.76	91.48	93.77	88.4
5	95.46	84.74	90.09	87.86	74.99	85.51	89.96	80.38
6	85.78	73.08	90.6	90.11	79.64	99.98	99.98	88.17
7	82.87	80.78	98.76	93.99	91.45	94.63	92.06	101.24
8	55.57	85.57	95.73	101.96	100.8	85.87	106.88	104.04
9	68.89	73.38	92.79	91.59	76.67	94.37	97.92	90.06
10	67.73	99.73	88.12	78.05	96.91	91.96	91.22	91.01
11	75.19	89.08	95.43	91.98	95.86	95.91	95.59	94.05
12	88.71	110.32	85.72	96.45	89.71	75.94	98.85	92.15
13	67.37	73.9	72.18	95.65	98.3	73.98	89.16	83.32
14	85.96	94.96	97.18	96.99	72.22	89.55	84.39	79.42
15	81.89	73.88	93.91	92.44	78.64	92.77	94.43	90.56
16	82.45	72.97	94.8	93.59	78.26	94.85	97.77	89.67
17	88.72	81.8	84.79	82.08	88.37	79.77	88.26	89.19
18	63.55	57.37	90.35	77.7	81.8	91.01	91.56	87.61
19	71.35	80.23	101.33	76.89	82.04	73.76	88.23	76.14
20	91.34	82.53	90.25	93	87.62	96.67	97.35	85.67
21	94.18	97.94	89.11	86.05	91.64	90.39	99.16	107.62
22	99.89	91.88	93.81	92.52	87.69	103.64	99.62	103.69
23	83.9	90.21	92.54	85.56	86.77	101.94	92.8	97.38
24	66.86	75.82	88.2	84.61	83.52	96.24	91.23	87.41
25	87.98	94.03	101.73	94.49	74.83	102.25	106.74	91.47
26	86.02	85.15	93.42	94.17	78.63	91.87	99.35	90.99
27	66.26	93.27	91.69	89.97	70.75	89.97	102.87	77.94
28	59.23	88.11	99.05	106.98	88.12	109.89	89.88	92.12
29	83.17	99.33	88.23	83.23	70.33	83.73	94.28	75.81
30	75.03	90.32	97.25	94.47	99.65	102.74	93.77	87.91

Table S3. (a) Factors examined in the screening phase of HPLC-UV separation conditions (full factorial design), (b) Matrix of the design, (c) The corresponding experimental response values for factorial design.

Table S3 (a)

Independent factors	Levels		
	Level (-1)	Level (0)	Level (+1)
A- ACN%	10	15	20
B- Column temperature (°C)	20	25	30
C- Flow rate (mL min ⁻¹)	0.8	1	1.2

Table S3 (b)

Std order	Run order	Experimental design			Experimental set-up		
		Factor 1	Factor 2	Factor 3	ACN%	Temp. °C	Flow rate (mL min ⁻¹)
5	1	-1	-1	1	10	20	1.2
1	2	-1	-1	-1	10	20	0.8
8	3	1	1	1	20	30	1.2
2	4	1	-1	-1	20	20	0.8
4	5	1	1	-1	20	30	0.8
7	6	-1	1	1	10	30	1.2
9	7	0	0	0	15	25	1
6	8	1	-1	1	20	20	1.2
3	9	-1	1	-1	10	30	0.8

Table S3 (c)

Run order	Responses					
	Rt(TMP)	Rt(CST)	Rs(TMP-CIP)	Rs(CIP-CTX)	Rs(CTX-DOX)	Rs(DOX-TYL)
1	3.69	13.88	1.12	4.14	5.56	4.60
2	4.96	15.20	2.31	6.14	3.85	3.96
3	1.86	12.88	1.11	4.98	3.20	5.02
4	2.22	14.29	1.92	10.42	2.42	4.51
5	2.22	14.12	3.43	6.20	3.17	4.12
6	3.54	13.78	2.66	3.87	5.25	5.48
7	3.03	13.85	2.02	5.83	4.89	5.21
8	1.87	13.00	1.45	5.79	2.53	3.99
9	4.86	15.08	1.95	5.99	4.34	4.07

Table S4. CCD for the HPLC response optimization. (a) Matrix of the design, (b) the corresponding experimental response values**Table S4 (a)**

Std order	Run order	Experimental design			Experimental set-up		
		Factor 1	Factor 2	Factor 3	ACN%	Temp. °C	Flow rate (mL min ⁻¹)
4	1	1	1	-1	20	30	0.8
15	2	0	0	0	15	25	1
13	3	0	0	-1.68	15	25	0.66
16	4	0	0	0	15	25	1
14	5	0	0	1.68	15	25	1.34
19	6	0	0	0	15	25	1
6	7	1	-1	1	20	20	1.2
7	8	-1	1	1	10	30	1.2
1	9	-1	-1	-1	10	20	0.8
18	10	0	0	0	15	25	1
2	11	1	-1	-1	20	20	0.8
20	12	0	0	0	15	25	1
8	13	1	1	1	20	30	1.2
5	14	-1	-1	1	10	20	1.2
11	15	0	-1.68	0	15	16.59	1
17	16	0	0	0	15	25	1
9	17	-1.68	0	0	6.59	25	1
3	18	-1	1	-1	10	30	0.8
12	19	0	1.68	0	15	33.41	1
10	20	1.68	0	0	23.41	25	1

Table S4 (b)

Run order	Responses					
	Rt(TMP)	Rt(CST)	Rs(TMP-CIP)	Rs(CIP-CTX)	Rs(CTX-DOX)	Rs(DOX-TYL)
1	2.04	14.21	3.42	6.23	3.94	4.12
2	3.15	13.96	2.91	6.08	5.27	4.27
3	4.43	15.23	2.73	5.45	3.69	4.26
4	3.14	12.88	2.51	5.64	5.72	4.70
5	2.42	13.16	1.86	3.35	5.80	5.28
6	3.10	14.00	2.65	5.62	5.82	5.10
7	1.90	12.99	0.86	3.75	2.65	4.08
8	3.52	13.72	2.32	3.9	7.40	5.71
9	5.12	14.82	2.95	5.00	5.65	3.72
10	3.01	13.86	2.02	5.21	5.01	5.10
11	2.78	14.84	0.57	6.42	2.56	4.32
12	2.99	13.83	2.50	5.83	5.12	5.02
13	1.92	12.87	1.15	3.79	3.01	5.01
14	3.6	13.82	3.32	4.08	6.69	4.88
15	3.16	13.93	2.51	5.76	5.91	4.28
16	3.03	13.85	2.02	5.83	4.89	5.21
17	4.76	15.58	2.74	3.81	6.85	5.26
18	4.76	14.92	2.02	5.26	4.27	4.05
19	3.03	12.84	3.01	5.10	4.31	5.10
20	1.65	13.19	3.01	4.62	2.03	3.71

Table S5. ANOVA results for FFD for screening of the factors affecting the extraction procedure. A 5% level of significance was desired.

Factors	R% (TMP)		R% (CIP)		R% (CTX)		R% (DOX)		R% (TYL)		R% (SMZ)		R% (FLU)		R% (CST)	
	F	p*														
Model	9.79	0.0454	261.19	0.0473	24.88	0.0391	69.62	0.0142	22.2	0.0437	86.93	0.0114	50.07	0.0197	22.01	0.044
A- MeoH																
amount (mL)	18.29	0.0235	557.23	0.027	16.42	0.0559	33.81	0.0283	3.28	0.2119	16.05	0.057	37.74	0.0255	60.21	0.0162
B- ACN																
amount (mL)	13.52	0.0348	60.66	0.0813	3.93	0.1858	80.9	0.0121	0.37	0.6071	63.07	0.0155	28.66	0.0332	4.42	0.1701
C- Formic acid %																
Time (min)	3.57	0.1552	1.98	0.3934	31.08	0.0307	125.73	0.0079	58.62	0.0166	290.28	0.0034	53.71	0.0181	10.82	0.0813
R ² _{adj}	0.9289		0.9994		0.9446		0.9800		0.9381		0.9840		0.9723		0.9375	

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

R²_{adj}: coefficient of determination.

Table S6. ANOVA results for CCD for optimization of the factors affecting the extraction procedure. A 5% level of significance was desired.

Factors	R% (TMP)		R% (CIP)		R% (CTX)		R% (DOX)		R% (TYL)		R% (SMZ)		R% (FLU)		R% (CST)	
	F	p*														
Model	3.28	0.0273	2.61	0.0377	2.84	0.0455	3.25	0.0282	4.35	0.0083	5.51	0.0025	2.57	0.0400	4.250	0.0092
A-MeOH amount(mL)	1.41	0.2466	0.47	0.502	0.01	0.9209	0.04	0.8277	2.82	0.1056	0.01	0.9082	5.40	0.0346	5.50E-03	0.9415
B-ACN amount(mL)	0.05	0.8109	0.27	0.6122	10.62	0.0032	12.36	0.0017	0.03	0.858	20.44	0.0001	8.43	0.0109	5.10	0.0329
C-Formic acid%	6.36	0.0184	0.09	0.766	4.55E-03	0.9467	0.44	0.5144	13.47	0.0012	0.02	0.8733	0.93	0.3512	3.85	0.0609
D- Time (min)	5.28	0.0302	13.96	0.002	0.72	0.4036	0.15	0.7023	1.10	0.3043	1.58	0.2207	1.18	0.294	8.06	0.0089
R ² _{adj}	0.8932		0.8755		0.8623		0.9021		0.8845		0.9169		0.8721		0.8855	

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

R²_{adj}: coefficient of determination

Table S7. ANOVA results for factorial design for screening factors affecting HPLC-UV method. A 5% level of significance was desired.

Factors	Rt (TMP)		Rt (CST)		Rs (TMP-CIP)		Rs (CIP-CTX)		Rs (CTX-DOX)		Rs (DOX-TYL)	
	F	p*	F	p*	F	p*	F	p*	F	p*	F	p*
Model	119.00	<0.0001	13.10	<0.0001	16.03	<0.0001	27.69	<0.0001	37.14	<0.0001	115.66	<0.0001
A- ACN%	417.71	<0.0001	38.83	<0.0001	0.054	0.8188	42.02	<0.0001	153.66	<0.0001	7.13	0.0156
B- Temp (°C)	0.35	0.5612	0.46	0.5046	17.59	0.0006	23.52	0.0002	6.71	0.0184	95.81	<0.0001
C- Flow rate (mL min ⁻¹)	57.93	<0.0001	82.22	<0.0001	33.98	<0.0001	79.66	<0.0001	19.95	0.0003	205.53	<0.0001
R ² _{adj}	0.9616		0.9978		0.8518		0.9072		0.8871		0.9614	

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

R²_{adj}: coefficient of determination.

Table S8. ANOVA results for CCD for optimization of HPLC-UV method. A 5% level of significance was desired.

Factors	Rt (TMP)		Rt (CST)		Rs (TMP-CIP)		Rs (CIP-CTX)		Rs (CTX-DOX)		Rs (DOX-TYL)	
	F	p*	F	p*	F	p*	F	p*	F	p*	F	p*
Model	101.19	< 0.0001	14.62	< 0.0001	3.5	0.0278	17.78	< 0.0001	13.48	< 0.0001	6.95	0.0033
A- ACN%	679.2	< 0.0001	14.19	0.0017	3.74	0.0752	7.99	0.018	64.05	< 0.0001	4.99	0.0402
B-Temp (°C)	6.99	0.0246	2.32	0.1474	0.91	0.3573	1.01	0.3378	0.42	0.527	4.51	0.0496
C- Flow rate (mL min ⁻¹)	187.49	< 0.0001	27.35	< 0.0001	1.67	0.2193	86.82	< 0.0001	7.61	0.0163	11.35	0.0039
R ² _{adj}	0.9794		0.9327		0.9174		0.9412		0.8615		0.8659	

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

R²_{adj}: coefficient of determination.

Table S9. The system suitability test results of the developed HPLC-UV method for determination of TMP, CIP, CTX, DOX, TYL, SMZ, FLU and CST.

Compound	Retention time (min)	Capacity factor (K')	Selectivity (α) ^a	Resolution (R_s) ^b	Tailing factor
TMP	4.16	1.56	1.12 _(a1)	2.71 _(b1)	1.13
CIP	4.48	1.76	1.21 _(a2)	4.78 _(b2)	1.12
CTX	5.08	2.13	1.30 _(a3)	5.88 _(b3)	1.05
DOX	6.12	2.77	1.18 _(a4)	2.54 _(b4)	0.93
TYL	6.96	3.29	1.18 _(a5)	3.25 _(b5)	1.04
SMZ	7.96	3.91	1.42 _(a6)	12.82 _(b6)	0.90
FLU	10.65	5.57	1.11 _(a7)	3.90 _(b7)	1.01
CST	11.71	6.22			1.09

The retention time of un retained peak is 1.62 min.

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

a₂, b₂: are α and R_s calculated for CTX-CIP.

a₃, b₃: are α and R_s calculated for DOX-CTX.

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

a₅, b₅: are α and R_s calculated for SMZ-TYL.

a₆, b₆: are α and R_s calculated for FLU-SMZ.

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

Table S10. Characteristic parameters of the calibration equations for the proposed HPLC-UV method for simultaneous determination of TMP, CIP, CTX, DOX, TYL, SMZ, FLU, and CST in Chicken sample.

Parameters	TMP	CIP	CTX	DOX	TYL	SMZ	FLU	CST
Calibration range ($\mu\text{g kg}^{-1}$)	30-300	30-300	30-300	30-300	40-300	30-300	30-300	30-300
Detection limit ($\mu\text{g kg}^{-1}$)	5	4	4	4	3	3	4	3
Quantitation limit ($\mu\text{g kg}^{-1}$)	16	13	14	13	11	10	15	12
Regression equation (Y) ^a :	330.66	1868.56	2546.88	2732.21	222.37	1171.12	1233.76	644.89
Slope (b)								
Standard deviation of the slope (S_b)	7.60	35.66	50.26	52.25	3.48	17.20	27.08	10.73
Relative standard deviation of the slope (%)	2.29	1.90	1.97	1.91	1.56	1.46	2.19	1.66
Confidence limit of the slope ^b	323.27- 338.05	1833.92- 1903.23	2498.05- 2595.72	2681.44- 2782.98	218.99- 225.76	1154.40- 1187.84	1154.40- 1187.84	634.46- 655.32
Intercept (a)	-1901.14	134312.73	209204.00	83548.74	1921.58	14956.28	10378.47	1990.69
Standard deviation of the intercept (S_a)	1192.91	5540.51	7808.15	8117.32	631.63	2672.61	4207.12	1684.24
Confidence limit of the intercept ^b	-3060.17- (-742.12)	128929.60- 139695.80	201617.60- 216790.30	75662.04- 91435.44	1307.90- 2535.27	12359.60- 17552.97	6290.87- 14466.07	354.30- 3627.08
Correlation coefficient (r)	0.9996	0.9997	0.9997	0.9996	0.9997	0.9998	0.9996	0.9998

^aY = 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 S11. Characteristic parameters of the calibration equations for LC-MS/MS method for simultaneous determination of TMP, CIP, CTX, DOX, TYL, SMZ, FLU, and CST in Chicken sample.

Parameters	TMP	CIP	CTX	DOX	TYL	SMZ	FLU	CST
Calibration range ($\mu\text{g kg}^{-1}$)	0.08-20	0.01-20	0.11-20	0.11-20	0.04-20	0.09-20	0.04-20	0.04-20
Detection limit ($\mu\text{g kg}^{-1}$)	0.02	0.05	0.03	0.03	0.01	0.02	0.01	0.01
Quantitation limit ($\mu\text{g kg}^{-1}$)	0.08	0.01	0.11	0.11	0.04	0.09	0.04	0.04
Regression equation (Y) ^a :								
Slope (b)	515.50	298.92	160.51	999.55	1121.90	257.28	5420.08	11706.07
Standard deviation of the slope (S_b)	6.10	2.14	2.55	15.97	6.84	3.42	35.16	69.46
Relative standard deviation of the slope (%)	1.18	0.71	1.59	1.59	0.60	1.33	0.64	0.59
Confidence limit of the slope ^b	509.56- 521.43	296.84- 301.00	158.03- 162.99	984.03- 1015.08	1115.25- 1128.55	253.96- 260.61	5385.92- 5454.24	11638.57- 11773.56
Intercept (a)	118.38	1.93	-20.68	-223.04	-250.04	153.12	-1512.52	951.16
Standard deviation of the intercept (S_a)	550.18	193.21	229.96	1439.41	616.45	308.51	3167.51	6257.68
Confidence limit of the intercept ^b	-416.17- 652.94	-185.79- 189.66	-244.11- 202.73	-1621.57- 1175.47	-848.99- 348.89	-146.62- 452.86	-4590.05- 1565.00	-5128.73- 7031.05
Correlation coefficient (r)	0.9999	0.9999	0.9997	0.9997	0.9999	0.9998	0.9999	0.9999

^aY = 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 S12. Intra-day and inter-day precision of the proposed HPLC-UV method determined by the recovery of TMP, CIP, CTX, DOX, TYL, SMZ, FLU, and CST from spiked Chicken tissues.

Concentration ($\mu\text{g Kg}^{-1}$)	Intra-day precision			Inter-day precision			
	Recovery % ^a	S.D	RSD %	Recovery % ^a	S.D	RSD %	
TMP	80	96.99 \pm 0.89	0.89	-3.01	96.16 \pm 1.24	1.29	-3.84
	100	98.82 \pm 0.77	0.78	-1.18	97.87 \pm 1.46	1.49	-2.13
	150	99.82 \pm 1.15	1.16	-0.18	97.32 \pm 0.83	0.85	-2.68
CIP	80	99.36 \pm 1.88	1.89	-0.64	95.06 \pm 1.88	1.97	-4.94
	100	98.04 \pm 1.77	1.80	-1.96	97.64 \pm 1.77	1.82	-2.36
	150	95.80 \pm 0.89	0.93	-4.20	97.79 \pm 1.79	1.83	-2.21
CTX	80	96.05 \pm 0.89	1.28	-3.95	100.05 \pm 2.36	2.35	0.05
	100	97.85 \pm 1.15	1.44	-2.15	98.77 \pm 0.79	0.79	-1.23
	150	95.22 \pm 2.07	2.17	-4.78	96.22 \pm 2.44	2.53	-3.78
DOX	80	96.06 \pm 2.20	2.29	-3.94	96.06 \pm 2.88	3.00	-3.94
	100	99.57 \pm 0.29	0.29	-0.43	98.06 \pm 1.27	1.30	-1.94
	150	98.04 \pm 0.76	0.77	-1.96	95.57 \pm 1.75	1.83	-4.43
TYL	100	96.07 \pm 1.46	1.51	-3.93	100.07 \pm 1.46	1.45	0.07
	150	95.66 \pm 2.10	2.19	-4.34	98.63 \pm 2.31	2.34	-1.37
	200	96.43 \pm 2.48	2.57	-3.57	95.41 \pm 0.91	0.95	-4.59
SMZ	50	97.66 \pm 0.55	0.56	-0.01	96.70 \pm 0.76	0.79	-3.30
	80	98.74 \pm 1.98	2.00	-2.46	98.41 \pm 1.76	1.79	-1.59
	100	99.77 \pm 0.07	0.07	-0.23	98.74 \pm 0.47	0.48	-1.26
FLU	50	97.54 \pm 0.54	0.55	-2.46	95.61 \pm 1.25	1.31	-4.39
	80	98.79 \pm 0.67	0.68	-1.21	97.69 \pm 0.99	1.01	-2.31
	100	98.30 \pm 0.80	0.81	-1.70	98.30 \pm 1.20	1.22	-1.70
CST	80	95.83 \pm 1.08	1.12	-4.17	99.53 \pm 0.71	0.71	-0.47
	100	95.67 \pm 2.57	2.69	-4.33	98.97 \pm 2.93	2.96	-1.21
	150	99.18 \pm 2.18	2.20	0.82	100.30 \pm 2.05	2.04	0.30

a: average of three determinations.

Table S13. Intra-day and inter-day precision of the proposed LC-MS/MS method determined by the recovery of TMP, CIP, CTX, DOX, TYL, SMZ, FLU, and CST from Chicken sample.

Concentration ($\mu\text{g Kg}^{-1}$)	Intra-day precision			Inter-day precision			
	Recovery% a	RSD	RE%	Recovery% a	RSD	RE%	
	$\pm \text{S.D}$	%		$\pm \text{S.D}$	%		
TMP	5	98.23 \pm 1.23	1.25	-1.77	98.23 \pm 2.03	2.06	-1.77
	10	99.72 \pm 0.97	0.97	-0.28	97.89 \pm 1.89	1.93	-2.11
	20	99.21 \pm 2.15	2.16	-0.79	100.12 \pm 3.12	3.11	0.12
CIP	5	98..32 \pm 0.88	0.89	-1.68	98.41 \pm 2.87	2.91	-1.59
	10	97.06 \pm 2.30	2.36	-2.94	99.20 \pm 0.86	0.86	-0.8
	20	98.89 \pm 1.25	1.26	-1.11	97.98 \pm 1.57	1.60	-2.02
CTX	5	99.98 \pm 2.65	2.65	-0.02	99.32 \pm 2.68	2.69	-0.68
	10	99.52 \pm 1.86	1.86	-0.48	98.76 \pm 1.87	1.89	-1.24
	20	97.87 \pm 1.78	1.81	-2.13	98.68 \pm 3.42	3.46	-1.32
DOX	5	100.10 \pm 2.54	2.53	0.1	98.23 \pm 1.98	2.01	-1.77
	10	99.87 \pm 1.24	1.24	-0.13	99.55 \pm 2.24	2.25	-0.45
	20	99.05 \pm 1.34	1.35	-0.95	98.57 \pm 1.87	1.89	-1.43
TYL	5	100.06 \pm 2.56	2.55	0.06	98.36 \pm 2.65	2.69	-1.64
	10	99.54 \pm 2.41	2.42	-0.46	99.54 \pm 2.87	2.88	-0.46
	20	99.04 \pm 1.86	1.87	-0.96	98.78 \pm 1.95	1.97	-1.22
SMZ	5	98.66 \pm 0.79	0.80	-1.34	100.20 \pm 2.34	2.33	0.2
	10	97.89 \pm 0.98	1.00	-2.11	99.42 \pm 0.99	0.99	-0.58
	20	98.78 \pm 1.35	1.36	-1.22	98.77 \pm 1.74	1.76	-1.23
FLU	5	98.64 \pm 0.84	0.85	-1.36	97.89 \pm 2.51	2.56	-2.11
	10	99.35 \pm 1.57	1.58	-0.65	99.25 \pm 1.69	1.70	-0.75
	20	98.65 \pm 1.74	1.76	-1.35	100.03 \pm 3.04	3.03	0.03
CST	5	97.66 \pm 2.30	2.35	-2.34	99.54 \pm 3.21	3.22	-0.46
	10	98.21 \pm 3.01	3.06	-1.79	97.88 \pm 2.78	2.84	-2.12
	20	98.44 \pm 1.16	1.17	-1.56	98.23 \pm 2.42	2.46	-1.77

a: average of three determinations.

Table 14. Determination of TMP, CIP, CTX, DOX, TYL, SMZ, FLU and CST in laboratory prepared mixtures using the proposed (a) HPLC -UV (b) LC/MS/MS methods.

a Mix. No	Compound conc. (μg /Kg)								%Recovery							
									HPLC-UV							
	TMP	CIP	CTX	DOX	TYL	SMZ	FLU	CST	TMP	CIP	CTX	DOX	TYL	SMZ	FLU	CST
1	30	40	300	60	70	100	150	300	99.5	100.6	99.3	99.8	99.1	101.1	99.5	100.6
2	300	300	300	300	300	300	300	300	99.6	100.2	99.8	100.3	99.9	100.5	99.5	100.7
3	30	30	30	30	40	30	30	30	100.2	99.5	100.2	100.5	100.6	100.5	100.8	100.5
4	100	50	200	250	300	100	30	60	100.5	99.5	99.9	99.8	100.0	100.2	100.8	99.5
5	150	200	300	50	50	90	90	90	99.6	99.7	99.1	99.9	99.8	99.6	100.3	99.3
					Mean				99.88	99.9	99.66	100.06	99.88	100.38	100.18	100.12
					± S.D.				0.44	0.48	0.45	0.32	0.54	0.54	0.65	0.66

b Mix. No	Compound conc. (μg /Kg)								%Recovery							
									LC/MS/MS							
	TMP	CIP	CTX	DOX	TYL	SMZ	FLU	CST	TMP	CIP	CTX	DOX	TYL	SMZ	FLU	CST
1	0.08	0.01	0.2	0.2	0.04	0.09	0.04	0.04	100.9	99.8	100.4	99.7	99.8	100.3	99.1	100.6
2	2	4	1	1	1	2	1	1	99.4	99.2	100.5	99.6	100.6	99.8	99.5	100.8
3	3	10	1.5	1.5	1.2	4	0.09	0.09	100.7	99.1	99.3	100.9	99	99.3	99.4	99.8
4	6	1	0.5	0.5	4	5	2	2	100.5	100.2	99.6	100.5	99.7	100.7	100.8	99.1
5	20	0.05	10	10	20	20	20	20	99.8	100.7	99.1	99.6	100.7	100.4	100.9	100.3
					Mean				100.26	99.8	99.78	100.06	99.96	100.1	99.94	100.12
					± S.D.				0.63	0.67	0.64	0.60	0.70	0.55	0.84	0.68