Supplementary Data

Development of a Nanocluster-Based Platform for Determination of Sofosbuvir

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Optimization of reaction conditions by experimental design

In order to optimize the reaction conditions, a CCD with four independent parameters was used. The coded and corresponding uncoded values are given in Table 1S. This experimental design includes 27 experiments ($N=2^k + 2k + n_o$), in which 'k' is the number of independent variables and n_o is the number of replication at the center point of the individual variables. Table 2S summarized the 4-factor CCD matrix and the obtained experimental results. After running the 27 experiments, the data obtained from the CCD was regressed by using response surface method. The obtained data were correlated by the following second-order polynomial model:

$$\boldsymbol{Y} = b_0 + b_1\boldsymbol{X_1} + b_2\boldsymbol{X_2} + b_3\boldsymbol{X_3} + b_4\boldsymbol{X_4} + b_{12}\boldsymbol{X_{12}} + b_{13}\boldsymbol{X_{13}} + b_{14}\boldsymbol{X_{14}} + b_{23}\boldsymbol{X_{23}} + b_{24}\boldsymbol{X_{24}} + b_{34}\boldsymbol{X_{34}} + b_{11}\boldsymbol{X_1^2} + b_{22}\boldsymbol{X_2^2} + b_{33}\boldsymbol{X_3^2} + b_{44}\boldsymbol{X_4^2} \quad (1)$$

In which, $\boldsymbol{Y}$ is the measured response, b_0 is the intercept, $\boldsymbol{X_i}$ is the levels of independent variables, b_1–b_4 are the linear coefficients, b_{12}–b_{34} show the interaction of investigated factors and b_{11}–b_{44} are the quadratic coefficients. The trained second-order polynomial equation in uncoded units by response surface analysis is summarized as:

$$Y = -368.099 + 99.162\,\boldsymbol{X_1} + 5708.804\,\boldsymbol{X_2} + 9.459\,\boldsymbol{X_3} - 8.411\,\boldsymbol{X_1^2} - 0.108\,\boldsymbol{X_3^2} - 0.401\,\boldsymbol{X_4^2} + 1.400\,\boldsymbol{X_{14}} - 276.190\,\boldsymbol{X_{23}} - 1.179\,\boldsymbol{X_{24}} - 0.031\,\boldsymbol{X_{34}} \quad (2)$$

Analysis of the residuals of the regression model and the obtained lack of fit test are given in Table 3S showing that the second-order polynomial model provide an adequate illustration of the surface over the studied region. As can be seen, the regression model show a high value of coefficient of measurement ($R^2 = 97.65\%$). This implies that 97.65% of the variations for *Y* are explained by the independent variables and this also means that the model does not explain only about 2.35% of variations.

Table 1S

Coded and actual values of independent variables of the experimental design

Variables	Ranges and levels				
	−2	−1	0	+1	+2
pH (X_1)	3.0	5.0	7.0	9.0	11.0
[Buffer] (mol L^{-1}) (X_2)	0.001	0.0025	0.004	0.0055	0.007
Cu NCs volume (µL) (X_3)	7.5	20.625	33.75	46.875	60.0
Time (X_4)	1	5.75	10.5	15.25	20.0

Table 2S

The 4-factor central composite design matrix and the value of response function

Run	X_1	X_2	X_3	X_4	Response	
					Experimental	predicted
1	-1	1	-1	-1	91	85
2	0	0	-2	0	45	50
3	1	-1	-1	-1	48	46
4	0	0	0	-2	90	93
5	1	1	1	-1	72	74
6	1	-1	-1	1	86	81
7	-1	-1	1	-1	121	119
8	0	-2	0	0	*	163
9	-2	0	0	0	19	24
10	1	1	1	1	70	69
11	0	0	2	0	89	88
12	-1	1	1	-1	100	105
13	1	1	-1	-1	48	54
14	1	-1	1	-1	82	87
15	-1	-1	-1	-1	70	77
16	0	0	0	0	163	152
17	1	1	-1	1	80	73
18	-1	-1	1	1	101	100
19	0	0	0	2	93	93
20	-1	-1	-1	1	91	81
21	1	-1	1	1	105	100
22	-1	1	-1	1	76	73
23	0	0	0	0	143	152
24	2	0	0	0	-5	-6
25	-1	1	1	1	80	69
26	0	0	0	0	148	152
27	0	2	0	0	*	142

* These results are considered as outliers and are excluded for modeling calculations.

Table 3S

Analysis of variance (ANOVA) for the response surface quadratic model obtained from CCD

Source of variations	Sum of squares	Degrees of freedom	Adjusted mean square	*F*	*P*
Model	32527.3	10	3252.7	58.14	0.000
Linear	4115.1	3	1371.7	24.52	0.000
A	1457	1	1457	26.04	0.000
B	473.1	1	473.1	8.46	0.011
C	2185	1	2185	39.06	0.000
Square	26117.9	3	8706	155.61	0.000
A*A	25020.3	1	25020.3	447.22	0.000
C*C	8475.3	1	8475.3	151.49	0.000
D*D	4233.3	1	4233.3	75.67	0.000
2-Way Interaction	2294.3	4	573.6	10.25	0.000
A*D	976.6	1	976.6	17.46	0.001
B*C	473.1	1	473.1	8.46	0.011
B*D	280.6	1	280.6	5.01	0.042
C*D	564.1	1	564.1	10.08	0.007
Error	783.2	14	55.9		
Lack-of-Fit	566.6	12	47.2	0.44	0.857
Pure Error	216.7	2	108.3		
Total	33310.6	24			

[a] Not statistically significant ($p>0.05$).

R^2= 97.65%, Adj. R^2= 95.97 %.