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Original Article

THE OPTIMIZATION OF HPLC FOR QUANTITATIVE ANALYSIS OF ACID ORANGE 7 AND SUDAN II IN COSMETIC PRODUCTS USING BOX BEHNKEN DESIGN

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ABSTRACT

Objective: The objective of this study was to optimize high-performance liquid chromatography (HPLC) method for the determination of acid orange 7 (AO7) and sudan II (SII) in blusher product based on response surface methodology using box behnken design (BBD) approach.

Methods: Some factors responsible for HPLC separation including column temperature, mobile phase composition, flow rate were optimized using BBD. The responses evaluated were peak area, retention time, and tailing factor. AO7 and SII in blusher product has different properties, therefore both analytes were analysed using C18 column (Thermo Synergy Gold 250 mm x 4.6 mm i.d.,5 µm) using Shimadzu LC 20AD chromatograph equipped with photo-diode array (PDA) detector at 300-650 nm. The mobile phase used was acetonitrile-water (1:1 v/v), and acetonitrile composition was optimized at 35-50% for separation AO7 (ACN1), and 80-90% for SII (ACN2), delivered at the flow rate of 0.9–1 ml/min, using column temperature at 30-40 °C.

Results: BBD showed that separation of AO7 was influenced by the concentration of ACN1, flow rate and column temperature. These factors affected retention time, peak area, and tailing factor with peak area was the most significant. Tailing factor was not significantly affected by each factor, and retention time was slightly effected. Otherwise, Sudan II was affected by all these factors except ACN1. The optimal condition obtained based BBD was ACN1 43%, ACN2 90%, the flow rate of 0.9 ml/min and a column temperature of 40 °C.

Conclusion: BBD can be used to get optimum condition for analysis of AO7 and SII in blusher product.

Keywords: Acid orange 7, Sudan II, HPLC, BBD, Blusher

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INTRODUCTION

Dyes is the most important additive component in cosmetics industry to improve personal appearance [1]. Acid orange 7 (AO7) and sudan II (fig. 1) are azo dyes. AO7 is allowed in cosmetics product except if it was used around eyes. Sudan II (SII) is a forbidden dyes. Because of its similarity colour, AO7 is often replaced by sudan II [2]. AO7 and SII are harmful for longterm use [3, 4]. Therefore, analytical methods for analysing AO7 and SII must be developed in order to ensure the cosmetic safety.

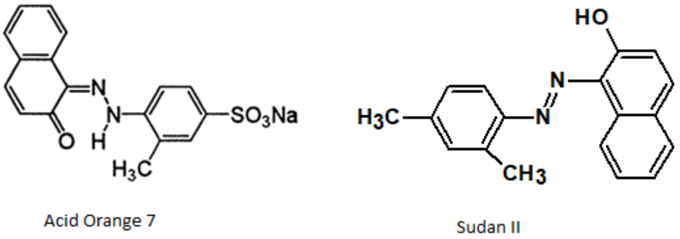


Fig. 1: The chemical structures of acid orange 7 (AO7) and sudan II (SII)

Different solubility of AO7 and SII make big challenge in the separation of both compounds simultaneously. Some methods have been reported for determination of AO7 and SII individually which are included ELISA, [5], UV-Vis [6], FTIR [7], HPTLC [8], LC/GC-MS [9, 10], HPLC [11-15]. These methods consume time and not effective, therefore optimization method HPLC for simultaneous analysis of AO7 and SII using experimental design is very interesting. Experimental design can predict optimum condition in short experiment and time. This design can determine the correlation between factors and respons (output) that are resulted in the experiment process [16].

MATERIALS AND METHODS

Blush products were obtained from local markets in Yogyakarta. Reference standars of acid orange 7 (CI 15510, Control Number: BR0114304), sudan II (CI 12140, Control Number: 113034) were acquired from the national agency of drug and food control (NADFC) of Republic of Indonesia. All solvents used for mobile phase were of HPLC grade and obtained from E. Merck (Darmstadt, Germany). Aquabidest was obtained from Ikapharmindo (Indonesia).

Preparation of reference standards

An approximately of 5.00 mg of each AO7 and SII was accurately weighed using analytical balance (Metler Toledo MX5) with a sensitivity of 0.01 mg and was added into volumetric flask 5 ml. AO7 was dissolved in 3 ml methanol, sonicated using sonicator (Elma ultrasonic, Germany) for 5 minute, and made to volume with methanol (5 ml) to get the solution with the concentration of 1000 µg/ml. SII was dissolved in 1 ml acetonitrile, added with 2 ml methanol, sonicated for 5 minute and made to volume 5 ml with methanol to get the solution with the concentration of 1000 µg/ml.

Preparation of samples

An approximately of 100.0 mg of blush cosmetic products was accurately weighed using analytical balance (Metler Toledo MX5)with the sensitivity of 0.1 mg, added with 1.0 ml of each standard solutions (AO7 and SII), added with 1 ml acetonitrile, sonicated for 5 minute, and added with methanol to volume 5 ml. The solution was filtered with PTFE 0.45 µm. In HPLC vial, 125 µl of this solution was added with 875 µl of acetonitrile: methanol (1:1 v/v). The solution was injected into HPLC system.

HPLC instrumentation

AO7 and SII were analysed using chromatograph of Shimadzu LC 20AD chromatograph equipped with photo-diode array (PDA) (Shimadzu LC 20AD, M20A PDA Detector) at wavelength of 300-650 nm. Separation of analytes was performed using C18 column (Thermo Synergy Gold 250 mm x 4.6 mm i.d., 5 µm). The mobile phase was modified from method of determining sudan dyes [12], used acetonitrile-water as solvent (1:1 v/v), the composition of acetonitrile was optimized at 35-50% for separation AO7 (ACN 1), 80-90% for SII (ACN 2), delivered at flow rate of 0.9–1 ml/min, using column temperature of 30-40 °C.

Experimental design using BBD

Most experimental design technique used is based on response surface methodology (RSM), a optimization based on fit of a polynomial equation to data experiment [17, 18]. Symmetrical design of RSM, namely central composite design (CCD) and box behnken design (BBD) are frequently used in HPLC method optimization because they can resolve HPLC separation-related problems which the number of factors is higher than 2 [18]. CCD and BBD have difference of selection experimental point, variables number, as well as number of run and block [19]. In this study, separation of AO7 and SII, four factors, also known as independent variables namely acetonitrile concentration for separation of AO7 (ACN1)(X1), acetonitrile concentration for separation of SII (ACN2) (X2), flow rate (X3), and column temperature (X4) were used. While, the responses, known as dependent variables evaluated included retention time AO7 and SII (Y1 and Y2), peak area AO7 and SII (Y3 and Y4), tailing factor AO7 and SII (Y5 and Y6).

Data analysis

All experiments using BBD along with statistical parameters were performed using Design-Expert version 8.0.4.1. The responses evaluated were retention time, peak area, tailing factor. of AO7 and SII. Factors (independent variables) significantly affected the responses (dependent variables) if $R^2 \geq 0.8$ and Adjusted $R^2 > 0.8$. The difference between Predicted R^2 with the Adjusted R^2 must be less than 0.2. The confirmation of optimal method was performed using six injection replicates. The statistical test of independent t-test was used for comparing results obtained from BBD and from actual experiments was carried out using Minitab software version 17 (Minitab Corp., USA).

Table 1: Box-Behnken design using dependent variables of concentration of acetonitrile 1 (%) (X1), concentration of acetonitrile 2 (%) (X2), flow rate (X3) and column temperature (X4) with response variables of retention time AO7 (Y1), retention time AO7 (Y2), peak area AO7 (Y3), peak area SII (Y4), tailing factor AO7 (Y5) and tailing factor SII (Y6) used in HPLC optimization for separation of Acid Orange 7 (AO1) and sudan II (SII)

Std	Run	Dependent variables				Responses					
		Conc. ACN 1 (%) (X1)	Conc. ACN 2 (%) (X2)	Flow rate (ml/min) (X3)	Column Temp. (°C) (X4)	Retention time AO7 (Y1)	Retention time SII (Y2)	Area AO7 (Y3)	Area SII (Y4)	TF AO7 (Y5)	TF SII (Y6)
29	1	42.5	85	1	35	2.551	14.075	718647	761589	0.857	1.031
28	2	42.5	85	1	35	2.495	14.130	710606	761514	0.726	1.028
27	3	42.5	85	1	35	2.391	14.134	706873	761573	0.810	1.025
14	4	42.5	90	0.9	35	2.577	14.179	783104	826350	1.026	1.048
20	5	50	85	1.1	35	1.991	12.636	629380	691823	0.800	1.035
2	6	50	80	1	35	2.158	15.339	690442	773393	0.771	1.001
10	7	50	85	1	30	2.458	14.286	691074	761217	0.849	1.030
9	8	35	85	1	30	3.106	15.045	709254	760680	0.617	1.026
3	9	35	90	1	35	2.699	13.261	716202	743710	0.709	1.055
11	10	35	85	1	40	2.183	14.012	677197	760764	0.682	1.017
8	11	42.5	85	1.1	40	1.836	12.631	638040	691491	0.709	1.018
19	12	35	85	1.1	35	2.406	13.314	654414	686654	0.654	1.033
23	13	42.5	80	1	40	1.975	15.245	691065	770904	0.779	0.985
6	14	42.5	85	1.1	30	2.404	13.585	650290	692914	0.792	1.036
17	15	35	85	0.9	35	2.634	15.808	775329	846130	0.646	1.017
18	16	50	85	0.9	35	2.257	14.983	760805	840086	0.767	1.023
12	17	50	85	1	40	1.858	13.232	685957	760518	0.763	1.013
25	18	42.5	85	1	35	2.125	14.120	697755	758976	0.763	1.017
16	19	42.5	90	1.1	35	1.903	11.982	637067	676598	0.731	1.051
1	20	35	80	1	35	2.286	16.215	678082	770621	0.681	0.994
26	21	42.5	85	1	35	2.060	14.138	701493	759523	0.692	1.020
5	22	42.5	85	0.9	30	2.729	16.085	778922	842236	0.905	1.027
22	23	42.5	90	1	30	2.596	13.439	709574	744095	0.747	1.060
21	24	42.5	80	1	30	2.494	16.614	711806	772956	0.743	1.006
4	25	50	90	1	35	1.965	12.565	690365	743483	0.817	1.049
13	26	42.5	80	0.9	35	2.178	17.461	766545	857715	0.853	0.986
24	27	42.5	90	1	40	1.942	12.573	686926	745445	0.860	1.038
7	28	42.5	85	0.9	40	2.078	14.984	754334	843051	0.938	1.008
15	29	42.5	80	1.1	35	1.792	14.690	635316	702640	0.713	0.987

RESULTS AND DISCUSSION

HPLC is the most method used for separation of dyes because of its sensitivity, precision, accuracy, time efficiency, low cost and robust [20]. The different polarity between AO7 and SII might be cope by gradient elution method, therefore the separation of AO7 and SII are rather difficult. Reversed phase (C18) column did not retain AO7 in the high concentration of non-polar solvent, while the high concentration of polar solvent such as water could increase its binding with the stationary phase, therefore an experimental design approach was used. Box-behnken design (BBD) was used for HPLC separation of AO7 and SII. BBD was performed using 29 runs, applying 4 independent variables (factors) namely concentration of acetonitrile 1 (%) (X1), concentration of acetonitrile 2 (%) (X2), flow rate (X3) and column temperature (X4) along with response variables of retention time AO7 (Y1), retention time AO7 (Y2), peak area AO7 (Y3), peak area SII (Y4), tailing factor AO7 (Y5) and tailing factor SII (Y6). BBD using these factors and responses resulted during optimization were compiled in table 1.

Based on analysis of variance (ANOVA) results, the equation obtained using X_1, X_2, X_3, and X_4 as independent variables with the retention time of AO7 (Y_1) as response was:

Y_1= 6.4401–2.92 x $10^{-2}X_1$–1.33x$10^{-2}X_2$–1.77X_3–0.07X_4 (Adj. $R^2$0.7484) (Eq. 1)

The statistic results revealed that Adj. R^2obtained was<0.8, which indicated that the experimental model was not good fit using polynomial equation [21]. Difference Predicted R^2 with the Adjusted R^2 in all respons less than 0.2. Eq.1 informed that that variables of the concentration of acetonitrile 1 (%)(X1), the concentration of acetonitrile 2 (%) (X2), flow rate (X3) and column temperature (X4) have a negative effect on the retention time of AO7.

The contour plot showed an interaction between factors [21]. Contour plot of retention time SII along with 3D surface graph was shown in fig. 2. Based on ANOVA results, variables of X_1, X_2, X_3 and X_4, as well as an interaction between X_1 and X_3, X_1 and X_4, X_3 and X_4 in linear form, contributed significantly for response of Y_1 ($P<0.05$). But, the interaction between X_2 and X_1, X_3, X_4 did not contributed significantly to retention time of SII.

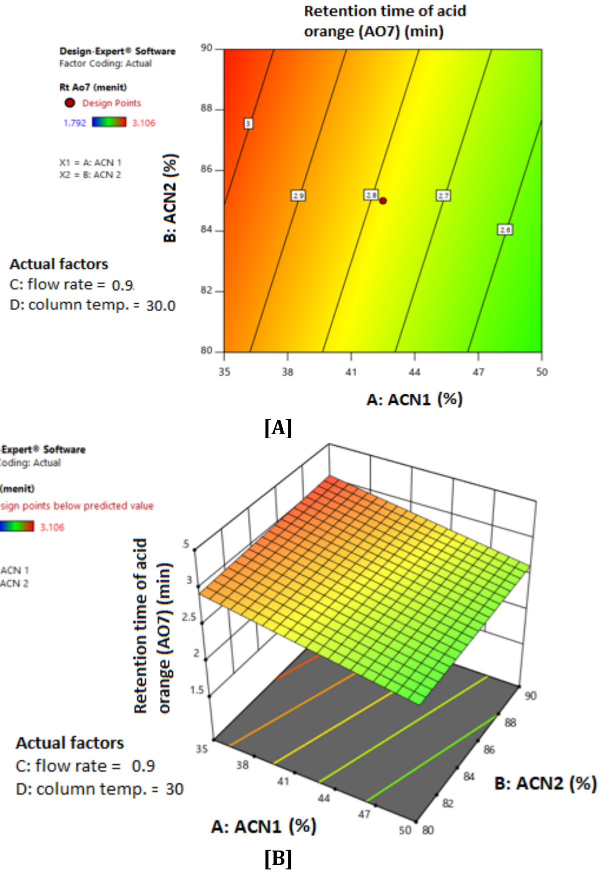


Fig. 2: The contour plot of Retention time of acid orange 7 (AO7) [A] and 3D surface graph of retention time of AO7 [B] as a results of variables of concentration of acetonitrile 1 (%) (ACN1), concentration of acetonitrile 2 (%) (ACN2), flow rate and column temperature

Similarly, the equation for retention time SII (Y_2) using multiple linear regression were:

$1/Y_2$=-0.237-9.80x$10^{-4}X_1$+5.19x$10^{-3}X_2$-1.93x$10^{-2}X_3$+7.30x$10^{-4}X_4$+ 04.00x$10^{-6}X_1X_2$+ 1.80x $10^{-4}X_1X_3$+ 4.95x$10^{-5}X_1X_4$+ 1.06x$10^{-3}X_2X_3$-0.000002.79x$10^{-6}X_2X_4$+4.9x$10^{-4}X_3X_4$+0.000006.21x$10^{-6}X_1^2$-2.90x$10^{-5}X_2^2$-0.018X_3^2-9.47x$10^{-6}X_4^2$(Adj. $R^2$of 0.9995) (Eq.2).

The contour plot along with along with 3D surface graph of the retention time of SII was shown in fig. 3. Statistic parameter of Y_2 revealed adjusted R^2 (Adj. R^2) was>0.8 (acceptable) [21] exhibiting that the experimental model was a good fit using the polynomial equation. Based on ANOVA results in variables of X_1, X_2, X_3 and X_4, as well as an interaction between X_2 and X_3, X_4,quadratic form of X_2 and X_4 contributed significantly for the response of Y_2 ($P<0.05$). The interactions of X_1-X_2, and X_3-X_4 were not significant to Y_2 response ($P>0.05$).

Equation 3 revealed the response of peak area Acid Orange 7 (Y_3). The statistic results for Y_3 informed that adj. $R^2$was>0.8. The variables of X_1, X_2, X_3 and X_4, as well as an interaction between X_1 and X_3, linear form of X_1 and X_3 contributed significantly for the response of Y_3 ($P<0.05$). The variables of X1 and X2 affected positively, meaning that the increased levels of concentration of acetonitrile 1 (%) (X1) and concentration of acetonitrile 2 (%) (X2) would increase peak area of AO7 (increased sensitivity), while the increased levels of flow rate (X3) and column temperature (X4) could decreased peak area. The contour plot along with along with 3D surface graph of the peak area of AO7 was shown in fig. 4.

Y_3=1.3738 x 10^{-3}+693.94 x $10^{-3}X_1$+833.03X_2–654443.33X_3–1956.68X_4 (Adj. $R^2$of 0.9463) (Eq. 3)

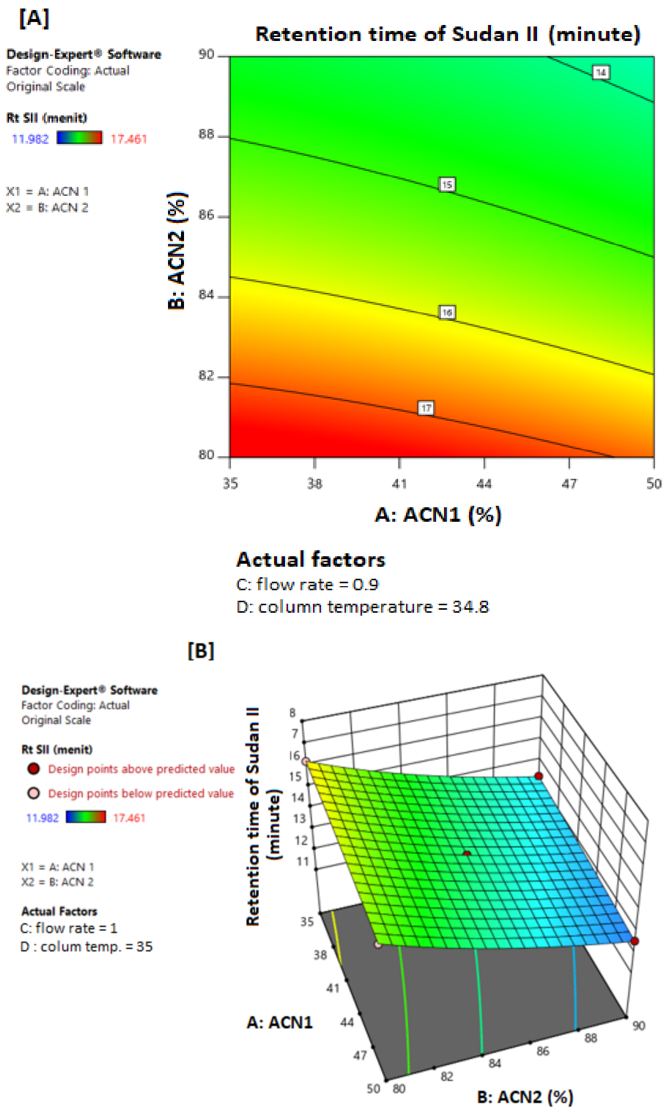


Fig. 3: The contour plot of retention time of Sudan II 7 (SII) [A] and 3D surface graph of retention time of SII [B] as a results of variables of concentration of acetonitrile 1 (%) (ACN1), concentration of acetonitrile 2 (%) (ACN2), flow rate and column temperature

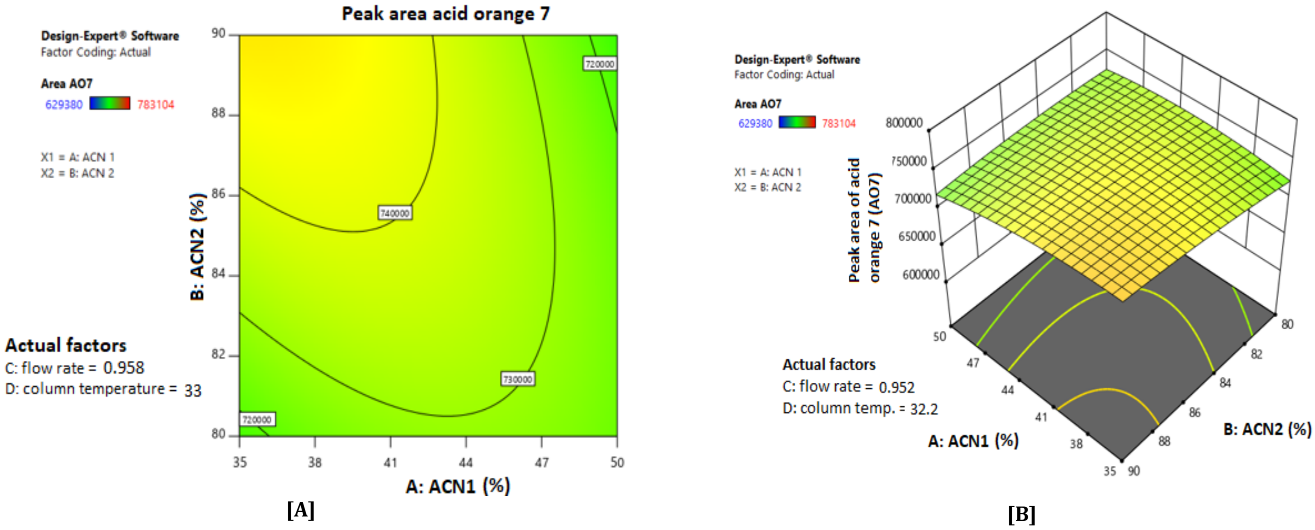
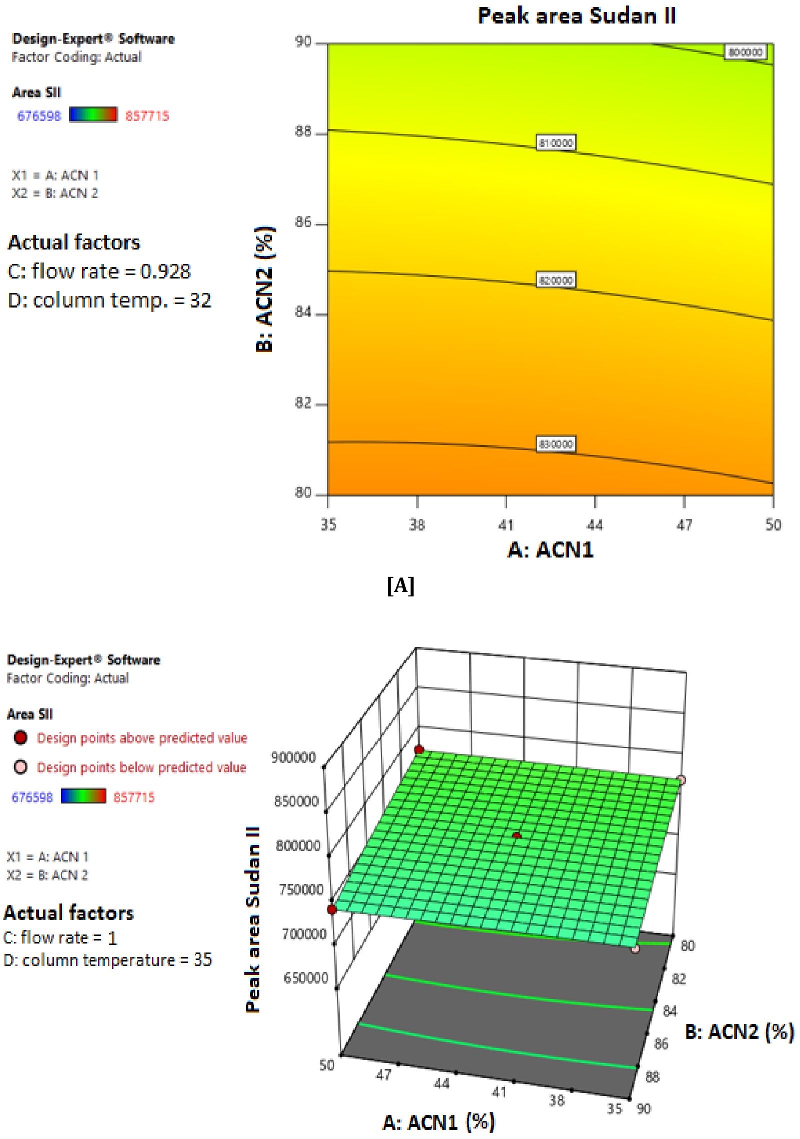


Fig. 4: The contour plot of peak area of acid orange 7 (AO7) [A] and 3D surface graph [B] as a results of variables of the concentration of acetonitrile 1 (%) (ACN1), a concentration of acetonitrile 2 (%) (ACN2), flow rate and column temperature

The equation 4 showed the correlation between the response of peak area of Sudan II (SII) and independent variables of X1, X2, X3 and X4 along with its interaction.

The statistic results for Y4 showed that Adj. R^2 obtained was in the acceptable limits [21]. The ANOVA results revealed that variables of X_1, X_2, X_3 and X_4, as well as an interaction between X_2-X_3, quadratic form X_2 and X_3 contributed significantly for the response of Y_4 ($P<0.05$). The interaction of X_1 with X_2, X_3, X_4 and between X_2 and X_4 did not contributed significantly to Y_4 response.

The contour plot along with along with 3D surface graph of the peak area of SII was shown in fig. 5.



[A]

[B]

Fig. 5: The contour plot of peak are of sudan II (SII) [A] and 3D surface graph of SII [B] as a results of variables of the concentration of acetonitrile 1 (%) (ACN1), the concentration of acetonitrile 2 (%) (ACN2), flow rate and column temperature

$Y_4=7.61 \times 10^{-8}+163.42X_1-14045.67X_2-76120.67X_3-160.42X_4-749.75X_1X_2+2803.25X_1X_3-195.75X_1X_4+1330.75X_2X_3+850.50X_2X_4-559.50X_3X_4-660.71X_1^2-2056.83X_2^2+6665.42X_3^2+238.29X_4^2$(Adj. R^2 of 0.9995)(Eq. 4)

The equations 5 and 6 corresponded to the response of tailing factor of AO7 (Y_5) and SII (Y_6). The statistic results for Y5 revealed that Adj. R^2 obtained was<0.8, which was not acceptable [18]. Based on ANOVA results, the variables of X_1, X_2, X_3 and X_4 has no interaction for all factors. Based on ANOVA results variables of X_1, X_2, X_3 and X_4, as well as an interaction between X_2-X_3, X_2-X_4,linear form of X_2 and X_3 contributed significantly for response of Y_6 ($P<0.05$).

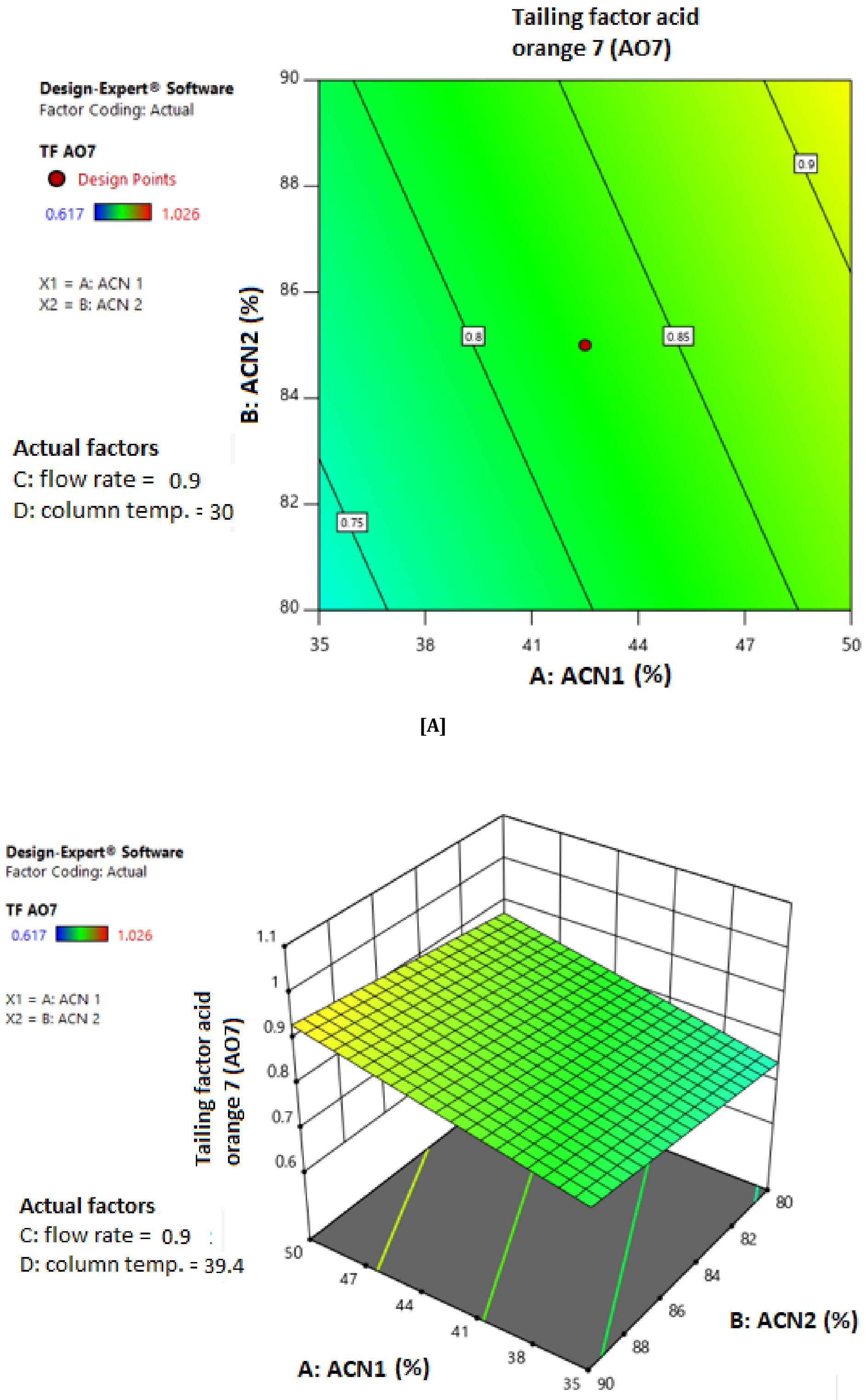
$Y_5=4.77x10^{-1}+8.64x10^{-3}X_1+5.83x10^{-3}X_2-0.61X_3+1.30x10^{-3}X_4$ (Eq. 5)

(Adj. R^2 of 0.3602)

$Y_6=5.53 \times 10^{-1}+1.00x10^{-4}X_1+5.7 \times 10^{-3}X_2+4.25 \times 10^{-2}X_3-1.77 \times 10^{-3}X_4$ (Eq. 6)

(Adj. R^2 of 0.9425).

Fig. 6 and fig. 7 showed the contour plot along with along with 3D surface graph of tailing factor of AO7 and SII.

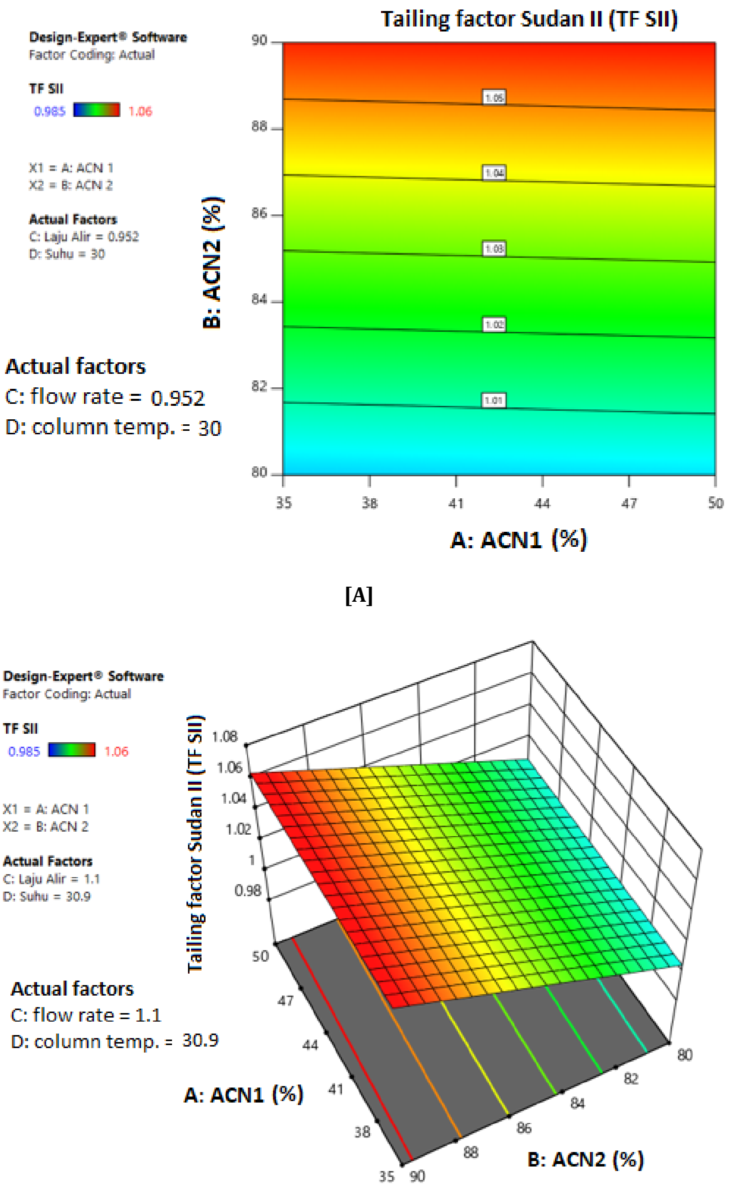


[A]

[B]

Fig. 6: The contour plot of tailing factor of acid orange 7 (AO7) [A] and 3D surface graph [B] as a results of variables of the concentration of acetonitrile 1 (%) (ACN1), the concentration of acetonitrile 2 (%) (ACN2), flow rate and column temperature

The optimum predicted conditions for separation AO7 and SII based on statistical results were as follows: ACN1 43%, ACN2 90%, flow rate of 0.9 ml/min and column temperature of 40 °C with the desirability of 0.818. It means that 81.80% data can be described by the selected model, the desired response would be reached easily [22]. The HPLC chromatogram obtained using this condition was shown in fig. 8. It is clear that both AO7 and SII were clearly separated using optimum condition suggested by BBD.



[A]

[B]

Fig. 7: The contour plot of tailing factor of sudan II (SII) [A] and 3D surface graph [B] as a results of variables of the concentration of acetonitrile 1 (%) (ACN1), the concentration of acetonitrile 2 (%) (ACN2), flow rate and column temperature

Fig. 8: Separation of acid orange 7 (AO7) and sudan II (SII) using HPLC condition as suggested by box-behnken design. See text for HPLC condition

CONCLUSION

BBD design can be used to get optimum condition for analysis of AO7 and Sudan II in blusher product. The optimum conditions suggested for separation AO7 and SII based on BBD was mobile phase containing ACN1 43% and ACN2 90% with flow rate of 0.9 ml/min, with column temperature of 40 °C.

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AUTHORS CONTRIBUTIONS

NBRP performed research activity, compiled data, and prepared manuscript. AR and SM designed research activities, prepared manuscript and made critical thinking on the manuscript.

CONFLICT OF INTERESTS

The authors have declared “no conflicts of interest with respect to the research, authorship, and/or publication of this article”.

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