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Optimization of HPLC Conditions for Analysis of Paracetamol in Jamu Pegal Linu in Pasar Besar Malang City Using Response Surface Methodology-Box-Behnken Design (RSM-BBD)

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ABSTRACT

Based on the Indonesian Ministry of Health Regulation No.7 in 2012, herbal medicine is disallowed to contain synthetic chemicals or isolated products with medicinal properties. However, from the findings of BPOM, there are still many herbal medicine producers who add medicinal chemicals to herbal medicine, one of which is the addition of paracetamol in jamu pegal linu. This study aimed to obtain optimum HPLC conditions for the analysis of paracetamol in jamu pegal linu using the Response Surface Methodology-Box-Behnken Design, ensure the HPLC method has met the validation parameters, and determine the presence and levels of paracetamol contained in jamu pegal linu sold in Pasar Besar, Malang City. HPLC conditions that were optimized were the percentage of methanol mobile phase in water, flow rate, and column temperature. The HPLC condition optimization results were obtained at a percentage of methanol mobile phase in water of 34.4%, mobile phase flow rate of 1 mL/min, and column temperature of 30°C. Analysis of paracetamol content of paracetamol in jamu pegal using HPLC and method validation with test parameters including selectivity (λmax 245 nm; paracetamol retention time ± 2.6 minutes; resolution ± 2.5), linearity (r2 = 0.9984), LOD (2.44 ppm), LOQ (7.40 ppm), accuracy (97-102%), and precision (0.26-0.69%). The analysis of paracetamol content in the three samples of jamu pegal linu analyzed showed that negative for paracetamol and fulfilled the regulation of the Indonesian Ministry of Health Regulation No.7 in 2012.

1. INTRODUCTION

Indonesia is a country that has abundant biological wealth, one of which is herbal plants [1]. Herbal plants or commonly called jamu by Indonesians, have been used since hundreds of years ago and are still an option for treatment today, one of the herbs that is often used is jamu pegal linu [2], [3]. Based on the Regulation of the Minister of Health of the Republic of Indonesia number 7 of 2012, jamu is disallowed to contain synthetic chemicals or isolated products with medicinal properties. Based on the website of the Ministry of Health of the Republic of Indonesia [4], there are still many irresponsible herbal medicine producers in Indonesia who add medicinal chemicals to herbal medicine, one of which is paracetamol in jamu pegal linu. This study aims to test the presence of paracetamol in jamu pegal linu products located in Pasar Besar Malang City as the market with the largest number of business places and traders in Malang City.

HPLC was chosen to analyze paracetamol content in herbal medicine because of its advantages in performing separation and analysis on highly complex mixtures quickly and efficiently [5]. The separation process using HPLC instruments is highly dependent on several components that can be regulated [6]. Therefore, it is necessary to optimize the HPLC condition components to get good analytical results. In this study, the HPLC components that were optimized were mobile phase, mobile phase flow rate, and column temperature.

Optimization in this research uses response surface methodology (RSM). The RSM design chosen is Box-Behnken Design because it can optimize three independent variables and can predict the optimum value with more efficient experiments

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so that it can shorten time and save costs [7]. After obtaining the optimum HPLC conditions, method validation needs to be carried out as an important step after method development which is a fundamental prerequisite for evaluating the ability of analytical methods to produce reliable analytical data [8].

2. MATERIALS AND METHODS

2.1 Materials

Jamu control ingredients, i.e. *Curcumae xantorrhizae rhizome*, *Curcumae domesticae rhizome*, and *Zingiberis officinalis rhizome*, were purchased from Pasar Besar, Malang City. The jamu control ingredients were obtained as a powder and identified by Herbal Materia Medica, Batu, East Java, Indonesia. Paracetamol as standard reference was purchased from Tokyo Chemical Industry. Pro-analysis grades of methanol was purchased from Fisher Chemical.

2.2 HPLC Instumentation dan Condition

Optimization and validation methods were carried out with the HPLC (LC-2030LT, Shimadzu, Japan) system equipped with Lab Solution software consisting of a pump, autosampler, column oven, and photodiode array (PDA) detector. Separations were carried out in a C18 column (4.6 mm x 150 mm, 5 µm). The injection volume was 20 µL and detection was monitored at a wavelength of 245 nm.

2.3 Preparation of Jamu Control and Jamu Control Containing Paracetamol Solution

3.5 grams of *Curcumae xanthorrhizae rhizome*, 3.5 grams of *Curcumae domesticae rhizome*, and 3 grams of *Zingiberis officinalis rhizome* [9] were weighed and mixed until homogenous. Jamu control was prepared by weighing 100 mg of the mixture, while jamu control containing paracetamol was prepared by weighing 75 mg of the mixture and 25 mg of paracetamol, then vortexed with ±30 mL methanol for ±15 minutes and add methanol until 100 mL in volumetric flask. A volume of 1.0 mL of each solution was transferred to 10 mL volumetric flasks using volumetric pipette and add methanol to a volume to achieve jamu control and jamu control containing paracetamol solution (25 µg/mL). Each solution was filtered using a 0.45 µm nylon syringe filter before being analyzed by HPLC.

2.4 Preparation of Standard Solution

10 mg of paracetamol was weighed and dissolved in 100 mL methanol. A volume of 5 mL of the solutions was transferred to 50 mL volumetric flask using volumetric pipette and add methanol to a volume to achieve standard solution with concentration of 100 µg/mL. Then, 1, 2, 3, 4, and 5 mL of the standard solution (100 µg/mL) was diluted with methanol in 10 mL volumetric flask to obtain working standard solutions with concentration of 10, 20, 30, 40, and 50 µg/mL. Each standard solution was filtered using a 0.45 µm nylon syringe filter before being analyzed by HPLC.

2.5 Preparation of Jamu Pegal Linu Sample Solution

100 mg of jamu pegal linu sample was weighed, then vortexed with ±30 mL methanol for ±15 minutes and add methanol until 100 mL in volumetric flask. A volume of 1.0 mL of each solution was transferred to 10 mL volumetric flasks using volumetric pipette and add methanol until the desired final volume. The solution was filtered using a 0.45 µm nylon syringe filter before being analyzed by HPLC.

2.6 Experimental Design for Optimization using RSM-BBD

RSM-BBD was used to optimize the HPLC conditions for the analysis of paracetamol in jamu pegal linu. The factors studied were the percentage of methanol mobile phase in water (A, %), flow rate (B, mL/min), and column temperature (C, °C) at three levels (coded -1, 0, and 1). The ranges of these factors are listed in Table 1. The responses evaluated in this study were peak area (Y_1), resolution (Y_2), tailing factor (Y_3), and theoretical plate (Y_4). Fifteen runs of experimental designs were obtained shown in Table 2. The solution used in the optimization was jamu control containing paracetamol solution.

RSM-BBD was performed using STATGRAPHICS Centurion 18 (Statgraphics Technologies Inc, USA). Analysis of variance (ANOVA) was used to determine the significance of each factor of interest. Excel Analysis Tool (Microsoft Office) was used to calculate data from non-factorial experiments.

Table 1. The studied factors in RSM-BBD

Factor	Level			Units
	-1	0	1	
A	30	60	90	%
B	0,5	0,75	1	mL/min
C	20	25	30	°C

Table 2. Box-Behnken Design Data Matrix and Responses

Run	A	B	C	Y_1	Y_2	Y_3	Y_4
1	-1,0	1,0	0,0	1962205	3,309	1,102	4670
2	1,0	0,0	-1,0	2974945	-	0,767	3738
3	0,0	0,0	0,0	2396598	0,757	-	6512
4	0,0	0,0	0,0	2395680	0,753	-	6498
5	-1,0	-1,0	0,0	1896523	-	-	2317
6	-1,0	0,0	-1,0	1340237	1,234	-	249
7	0,0	1,0	-1,0	2180624	-	0,780	5279
8	0,0	0,0	0,0	2652630	1,013	-	5939
9	0,0	-1,0	1,0	3858156	0,810	-	6904
10	0,0	-1,0	-1,0	4111573	1,014	-	6642
11	0,0	1,0	1,0	2174518	-	0,774	5195
12	1,0	1,0	0,0	2264351	-	0,878	3970
13	1,0	0,0	1,0	3012394	-	0,841	4853
14	1,0	-1,0	0,0	2551644	-	-	901
15	-1,0	0,0	1,0	1418622	1,808	-	933

A = Percentage of methanol mobile phase in water
B = Flow rate
C = Column temperature
Y_1 = Peak area
Y_2 = Resolution
Y_3 = Tailing factor
Y_4 = Theoretical plate

2.7 System Suitability Testing

The solution used in the system suitability test was paracetamol standard solution (30 µg/mL). The solution was injected six times into the HPLC system. System suitability parameters include peak area, retention time, resolution, tailing factor, theoretical plates, and percentage of relative standard deviation (%RSD).

2.8 Method Valid`ation

According to the ICH guidelines, the developed HPLC method was validated by determining the following parameters: accuracy, precision, selectivity, linearity, limit of detection (LOD), and limit of quantification (LOQ) [10].

a) Selectivity

The solution used in the selectivity test was paracetamol standard (30 μg/mL), jamu control, jamu control containing paracetamol, and jamu pegal linu sample solution. The similarity of the retention time and resolution of the chromatogram of all solutions were observed.

b) Linearity

Linearity was determined by making a calibration curve using paracetamol standard solutions with five different concentrations (10, 20, 30, 40, and 50 μg/mL). The calibration curve was obtained by plotting the concentration (μg/mL) against the peak area response of the paracetamol standard solution. Linearity was determined by correlation coefficient (r) using linear regression analysis.

c) LOD and LOQ

LOD and LOQ were determined by calculation using the standard deviation approach of the calibration curve (Equation (1) and (2)) [10].

$$\text{LOD} = \frac{3.3 \times \text{standard deviation of peak area}}{\text{slope of the calibration curve}} \quad (1)$$

$$\text{LOQ} = \frac{10 \times \text{standard deviation of peak area}}{\text{slope of the calibration curve}} \quad (2)$$

d) Accuracy and Precision

The solution used in the accuracy and precision test was jamu control containing paracetamol solution with three different of concentrations (80, 100, and 120%). The solution was prepared by weighing 90 mg of jamu control mixture powder and 10 mg of paracetamol, then vortexed with ±30 mL methanol for ±15 minutes and add methanol until 100 mL in volumetric flask. A volume of 2, 2.5, and 3 mL of the solutions was transferred to 10 mL volumetric flasks using volumetric pipette and add methanol to a volume to achieve jamu control containing paracetamol solution with concentration of 20, 25, and 30 μg/mL. Each concentration of jamu control containing paracetamol solution was made in three replications. All solution was filtered using a 0.45 μm nylon syringe filter before being analyzed by HPLC. Accuracy is represented by percent recovery, while precision is represented by %RSD calculated using equations (3) and (4)

$$\%\text{recovery} = \frac{\text{measured concentration}}{\text{actual concentration}} \quad (3)$$

$$\%\text{RSD} = \frac{\text{standard deviation}}{\text{mean}} \times 100\% \quad (4)$$

2.9 Analysis of paracetamol content in jamu pegal linu samples

The jamu pegal linu sample solution was injected into HPLC. The chromatogram was observed and the peak area that appears is calculated through the regression equation to the obtain concentration of paracetamol.

3. RESULTS AND DISCUSSION

3.1 Assessment of the effect of factors on the responses

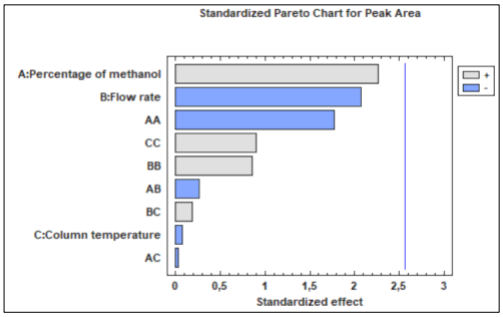
The effects of the factors and possible interactions with the response were evaluated by analysis of variance (ANOVA). The standardized Pareto chart, shown in Figure 1, allowing knowledge of the influential factors and their order of influence from a graphical point of view. Factors that have a significant influence on the response will be indicated by bars that cross the vertical line on the Pareto chart, confirming that the model appears to represent the experimental results at the 95% confidence limit [11].

On the peak area response, ANOVA (Table 3) and Pareto chart (Figure 1) showed that all factors had a p-value > 0.05, indicating that no factor has a significant influence on the peak area response. The factor of the percentage of methanol mobile phase in water has a positive influence on the peak area response, indicating that an increase in the percentage of methanol in water will increase the area response. This is in accordance with previous research, which shows that methanol has a higher eluation ability than water on the C18 stationary phase. The higher the percentage of methanol used, the better the elution and produce the maximum peak area [12]. Conversely, the flow rate and column temperature factors have a negative influence, indicating that an increase in the value of these factors will decrease the peak area response. Both of these are in accordance with previous research, which shows that increasing the high flow rate will damage the complete adsorption of the analyte on the column so that the separation that occurs is not optimal and produces a peak area that is not maximized. Increasing the temperature can cause a decrease in the viscosity of the mobile phase which leads to lower resistance to mass transfer and the resulting peak becomes more minimal [13].

On the resolution response, ANOVA (Table 3) and Pareto chart (Figure 1) showed that the percentage of methanol mobile phase in water factor had a p-value < 0.05, indicating that the factor has a significant influence on the resolution response. The percentage of methanol in water has a negative influence on the resolution response, indicating that increasing the percentage of methanol in water will decrease the response area. This is in accordance with previous research, which states that the addition of methanol which is a polar solvent causes a decrease in separation efficiency due to an increase in acid-base interactions between the solute and the mobile phase, thus causing a reduction in peak resolution [14].

Conversely, the flow rate and column temperature factors have a positive influence, indicating that increasing the value of these factors will increase the resolution response. Both of these are in accordance with previous studies, which show that increasing the flow rate can improve resolution because the solvent can interact more quickly with the solute and stationary phase resulting in an increase in separation efficiency which results in better peak resolution [13], [15]. High column temperature can increase resolution because it can reduce the viscosity of the mobile phase and increase the diffusion rate of the solvent so that the column efficiency increases. However, increasing the flow rate and column temperature too high will cause a decrease in resolution because it can cause peak broadening and reduce the interaction between the analyte and the mobile phase, so the use of optimal flow rate and column temperature is very important to optimize the separation [13].

A



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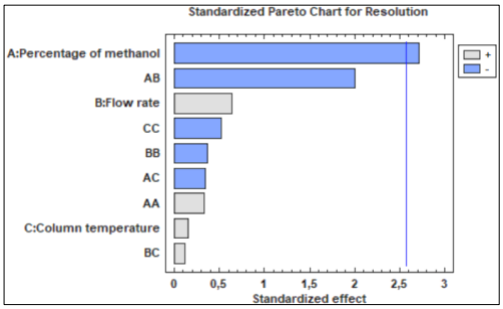

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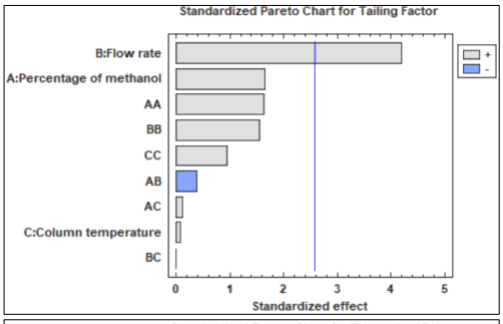

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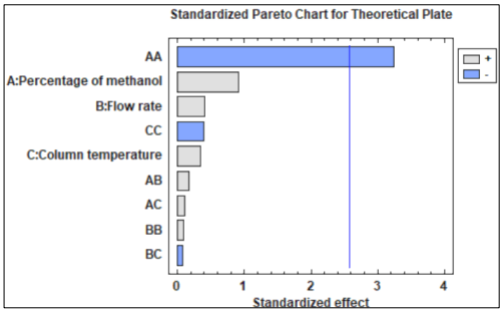

Fig. 1. Pareto Charts of Factor Effect on (A) Peak Area, (B) Resolution, (C) Tailing Factor, and (D) Theoretical Plate Responses

Table 3. ANOVA Results for the Models

Source of variation	p-Value from Full quadratic models			
	Y_1	Y_2	Y_3	Y_4
A: Percentage methanol mobile phase in water	0,07	0,04	0,16	0,40
B: Flow rate	0,09	0,55	0,01	0,70
C: Column temperature	0,94	0,88	0,94	0,75
AA	0,14	0,75	0,16	0,02
AB	0,80	0,10	0,72	0,87
AC	0,98	0,74	0,91	0,92
BB	0,43	0,73	0,18	0,93
BC	0,86	0,91	0,99	0,94
CC	0,41	0,62	0,38	0,71

Table 4. Model Fitting Results

Model Term	Full quadratic models			
	Y_1	Y_2	Y_3	Y_4
R-squared	74,47	71,39	83,75	70,37
p-value of lack of fit	0,11	0,07	0	0,06

On the tailing factor response, ANOVA (Table 3) and Pareto chart (Figure 1) showed that the flow rate factor had a p-value < 0.05, indicating that the factor had a significant influence on the tailing factor response. The three main factors have a negative influence on the tailing factor response, which indicates that an increase in the value of the factor will decrease the tailing factor response. The addition of methanol, which is a polar solvent, causes an increase in acid-base interactions between the solute and the mobile phase which makes the separation efficiency decrease and peak distortion occur, thereby increasing the tailing factor [14]. High flow rates can cause a decrease in column efficiency because the components in the column have less time to reach equilibrium. This causes band widening and individual particles of one compound become less dense, resulting in a higher tailing factor [16]. High column temperature can increase the tailing factor because the high temperature will make the stationary phase more hydrophobic resulting in peak broadening and reduced separation efficiency [17].

On the theoretical plate response, ANOVA (Table 3) and Pareto chart (Figure 1) showed that the quadratic factor of percentage of methanol mobile phase in water had a p-value < 0.05, indicating that the factor had a significant influence on the theoretical plate response. The three main factors have a negative influence on the tailing factor response, which indicates that an increase in the value of the factor will decrease the theoretical plate response. Increasing the amount of methanol in the mobile phase can make the polarity of the mobile phase increase, which causes more interaction between the stationary phase and the mobile phase. This increased interaction can shorten the retention period of the compound, resulting in a higher theoretical plate count [14]. Increasing the flow rate can increase the theoretical plate in chromatographic separation because a high flow rate can decrease the longitudinal diffusion factor, resulting in a lower plate height and causing more theoretical plates. High column temperature can increase the theoretical plate because high column temperature can reduce the viscosity of the mobile phase and increase the solvent diffusion rate resulting in an increase in column efficiency which results in a higher theoretical plate value [18].

3.2 Model Statistical Analysis

Regression model analysis is carried out to analyze the collected data and produce a relationship between responses and variables. Regression model analysis is carried out by connecting the observed response with the predicted response into a linear regression so that the coefficient of determination (r^2) value is obtained which is used to show the confidence that the regression model matches the analysis results. The predicted response value is determined through the mathematical

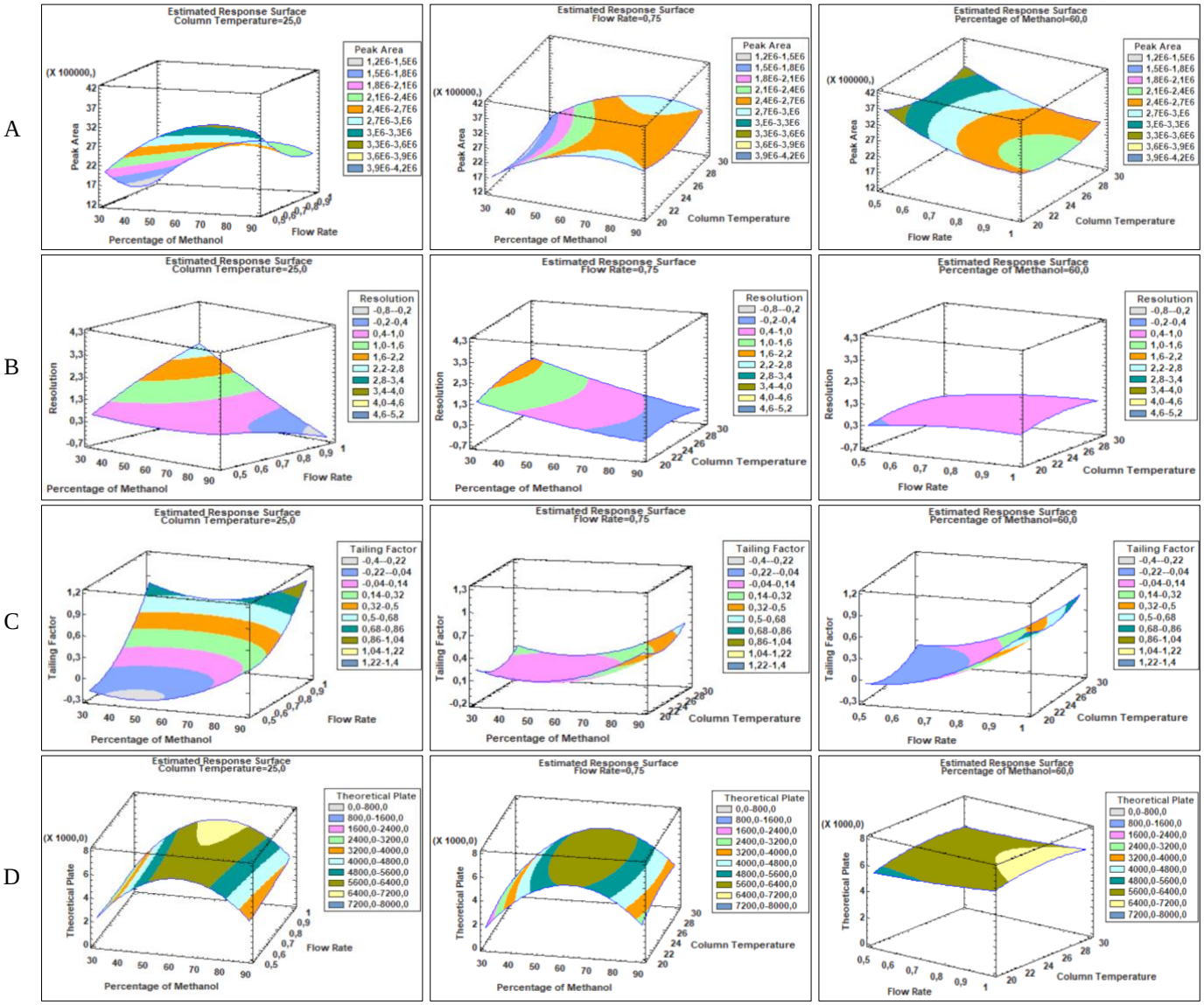

Fig. 2. Three-dimensional Response Surface Plots Showing the Relationship of the Factor to the (A) Peak Area, (B) Resolution, (C) Tailing Factor, and (D) Theoretical Plate Responses

equation obtained from the ANOVA results. The equation for each response was:

$$Y_1 = 11179800 + 108479A - 9429260B - 654046C - 670{,}90A^2 - 11765{,}8AB - 68{,}23AC + 4653700B^2 + 49462{,}2BC + 12349C^2 \quad (5)$$

$$Y_2 = -10{,}4904 + 0{,}06A + 10{,}16B + 0{,}49C + 0{,}0002A^2 - 0{,}11AB - 0{,}001AC - 2{,}55B^2 + 0{,}04BC - 0{,}01C^2 \quad (6)$$

$$Y_3 = 4{,}99 - 0{,}03A - 3{,}53B - 0{,}3C + 0{,}0003A^2 - 0{,}007AB + 0{,}0001AC + 3{,}85B^2 - 0{,}001BC + 0{,}006C^2 \quad (7)$$

$$Y_4 = -19559{,}6 + 447{,}10A - 1046B + 890{,}81C - 3{,}84A^2 + 23{,}87AB + 0{,}72AC + 1679{,}33B^2 - 69{,}2BC - 16{,}65C^2 \quad (8)$$

The coefficient of determination obtained from the regression model of all responses is 0.7037-0.8375, shown in Table 4. A linear regression model with a good coefficient of determination value shows confidence that the regression model fits the observed data if it has a coefficient of determination of more than 0.8 [19]. So this research has shown a regression model that is quite good and fit for the observed data. Additionally, the p-value in the lack of fit test is to assess the fit of the model to the response, whereas the p-value in the lack of fit test is said to fit well if it is more than 0.05 [20]. In this study, the p-value in three responses (peak area, resolution, and theoretical plate) is more than 0.05 (Table 4) so the models are fitted well, but the model in the tailing factor response is not fitted well.

3.3 Responses Optimization

Three-dimensional response surface plots (Figure 2) were made based on the predicted model to predict the relationship between factors and responses. RSM showed that the optimum peak area response (3800560) was obtained at a percentage of methanol in water of 75.44%, a flow rate of 0.5 mL/min, and a column temperature of 20°C. In the resolution response, RSM showed that the optimum resolution response (2.7) was obtained at a percentage of methanol in water of 30%, a flow

rate of 1 mL/min, and a column temperature of 27.66°C. In the tailing factor response, RSM showed the optimum tailing factor response (1) was obtained at a percentage of methanol in water of 89.21%, a flow rate of 0.99 mL/min, and a column temperature of 24.78°C. While in the theoretical plate response, RSM shows the optimum theoretical plate response (6784.31) is obtained at the percentage of methanol in water of 63.75%, flow rate of 1 mL/min, and column temperature of 26.05°C.

The results of a good HPLC analysis do not only depend on one response, therefore it is necessary to optimize based on all responses. Under these conditions, the desirability function is used to determine the most suitable condition from all responses. A desirability function close to 1 indicates that the response value is desirable or ideal [21]. The optimization results showed that the variable value to achieve the optimum value of the four responses (peak area, resolution, tailing factor, and theoretical plate) was obtained at the variable percentage of methanol in water of 34.4%, flow rate of 1 mL/min, and column temperature of 30°C with a desirability value of 0.2873. This shows that only 28.73% of the data can be explained by the model, so the model is not good enough to achieve the desired response.

3.4 System Suitability Testing

The system suitability test aims to verify that the detection sensitivity, resolution, and repeatability of the chromatographic system are adequate for the analysis to be performed [22]. Parameters used in the system suitability test are resolution (Rs), tailing factor (T), theoretical plate (N), and Relative Standard Deviation (RSD). Resolution shows the ability of the column to separate the two components into individual peaks, generally a good resolution value is Rs ≥ 2. The tailing factor shows the symmetry of the peaks, if there is tailing in chromatography, the shape of the chromatogram becomes asymmetrical. Tailing factor values are typically between 1.0-1.5 and values greater than 2 are not acceptable. The theoretical plate is a parameter to measure column efficiency [23], [24].

The results of the system suitability test conducted are listed in Table 5. Based on CDER, the requirements for the SST are to have a resolution value > 2, tailing factor ≤ 2, theoretical plate > 2000, and %RSD from repeated injection <1% [25] so that the results of the system suitability test carried out have met the requirements.

3.5 Method Validation

a) Selectivity

Selectivity tests are carried out by comparing retention times and spectra of the standard with other solutions. Data to ensure that the target compound has been separated from other compounds contained in the sample is resolution. Based on AOAC, a good resolution value is Rs > 1.5 [26]. In the selectivity test, a peak of paracetamol was obtained at a retention time of 2.644 (Figure 3). Only the jamu control containing paracetamol had a peak at that retention time. Then the chromatogram of the jamu control containing paracetamol was analyzed and the target peak was obtained at a retention time of 2.640 with a resolution value of 2.460 (Figure 4). Additionally, jamu control containing paracetamol had the same spectral shape with the standard (Figure 5). The resolution value of the target peak (paracetamol peak) meets the requirements set by AOAC so that the method used is selective for the test of paracetamol content in jamu pegal linu.

Table 5. System Suitability Results

Inject	Rt	Peak Area	Tailing Factor	Theoretical Plate	Resolution
1	2,649	2248240	1,141	4193	2,453
2	2,648	2239772	1,135	4188	2,464
3	2,650	2257452	1,132	4207	2,441
4	2,650	2260827	1,134	4219	2,442
5	2,649	2257072	1,134	4193	2,451
6	2,649	2250843	1,130	4202	2,445
%RSD		0,31%	0,30%	0,25%	0,32%

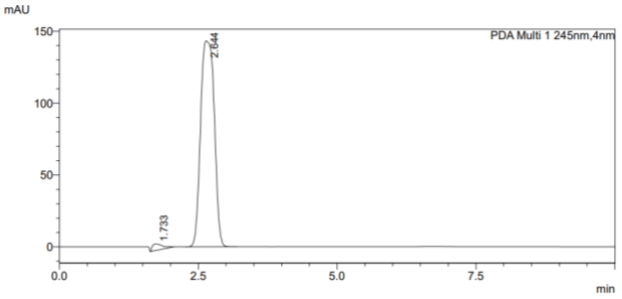

Fig. 3. Chromatogram of Paracetamol Standard

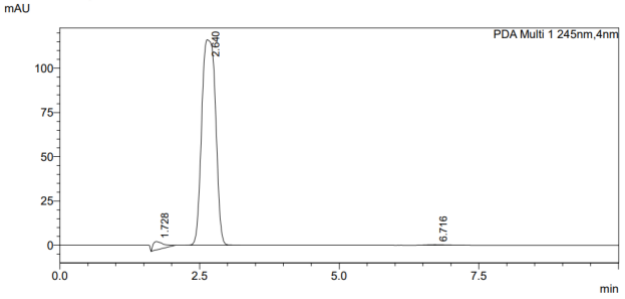

Fig. 4. Chromatogram of Jamu Control Containing Paracetamol

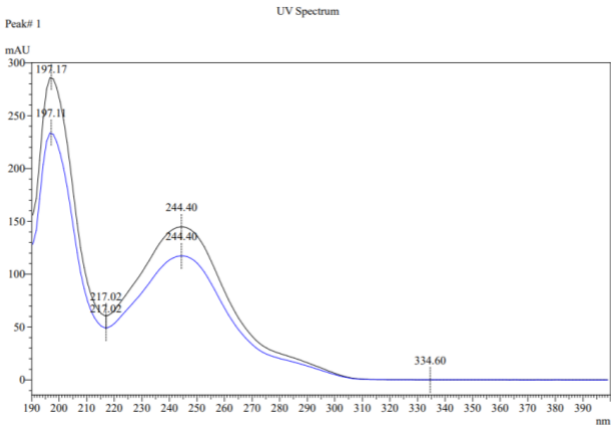

Fig. 5. Spectrum UV Overlay of Paracetamol Standard (Black) and Jamu Control Containing Paracetamol (Blue)

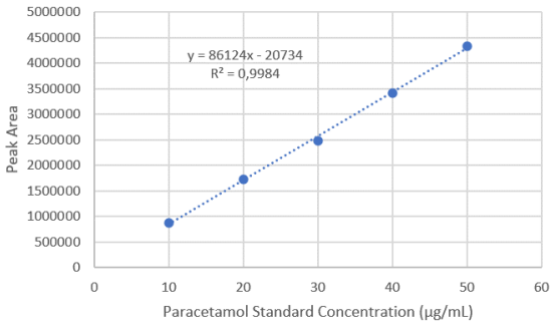

Fig. 6. Calibration Curve of Paracetamol Standard

b) Linearity

Determination of linearity is done by making a standard curve of the standard concentration with the measurement results. The calibration curve is shown in Figure 6. The regression equation obtained is y = 86124x-20734 with a correlation coefficient value of r = 0.9984. Based on AOAC, the requirement for a correlation coefficient on good linearity is r > 0.99 [26]. It can be concluded that the test has met the requirements and can be said to be linear.

c) LOD and LOQ

The LOD and LOQ values are obtained based on the calculation of the linearity equation (y = 86124x - 20734) using the standard deviation value of the regression line with a predetermined formula. The LOD and LOQ values obtained from the calculation results are LOD of 2.4431 ppm and LOQ of 7.4033 ppm. Low LOD and LOQ values indicate good method sensitivity [27].

d) Accuracy and Precision

Accuracy and precision tests were carried out using the recovery test method on the jamu control that had been made by adding paracetamol standards with a concentration range of 20 µg/mL as 80% concentration, 25 µg/mL as 100% concentration, and 30 µg/mL as 120% concentration. Each concentration was replicated three times. The peak area obtained from each concentration was then entered into the regression equation y = 86124x - 20734 to obtain the measured concentration of each test solution. In the accuracy and precision tests (Table 6), an average %recovery of 97-102% was obtained for the accuracy test and an average %RSD of 0.26-0.69% for the precision test. Based on AOAC, the acceptance requirements for accuracy and precision testing with 10% analyte concentration are 95-102% for %recovery and <1.5% for %RSD [26]. In this study, the accuracy and precision test results were obtained that met the requirements, so the method used in this study can be said to be accurate and precise.

3.6 Analysis of paracetamol content in jamu pegal linu samples

Analysis of paracetamol content was carried out on 3 samples of jamu pegal linu that had been obtained from traders in Pasar Besar Malang, which were labeled trader A, trader B, and trader C. Analysis of paracetamol content in jamu pegal linu samples was carried out using HPLC with HPLC conditions that had been optimized using the RSM-BBD method in this study. Furthermore, the chromatogram of each sample was observed, namely sample A (Figure 7), sample B (Figure 8), and sample C (Figure 9). In the test, the three samples tested did not show the presence of paracetamol compounds as indicated by the absence of paracetamol peaks with a retention time of ±2.64 minutes in the three sample chromatograms tested.

Based on the Regulation of the Minister of Health of the Republic of Indonesia Number 7 of 2012, herbal medicine is disallowed to contain synthetic chemicals or isolated products with medicinal properties. In this study, the three samples, namely samples A, B, and C, did not contain additional paracetamol medicinal chemicals. This shows that the three samples marketed in Pasar Besar Malang City meet Indonesian regulations and are safe for use by the public.

Table 5. Accuracy and Precision Test Results

Theoretical Conc.	Measured Conc.	%Recovery	Average % Recovery	%RSD
20	20,66	103%	102%	0,69%
	20,48	102%		
	20,32	102%		
25	25,33	101%	102%	0,26%
	25,44	102%		
	25,49	102%		
30	29,11	97%	97%	0,42%
	29,04	97%		
	28,83	96%		
Average Total			100%	0,45%

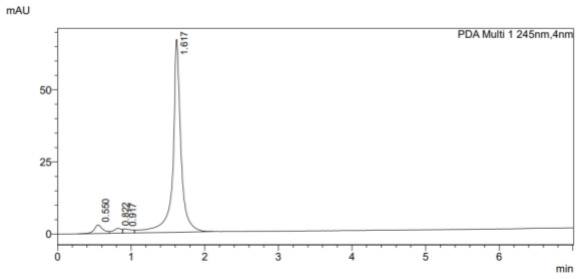

Fig. 7. Chromatogram of Sample A

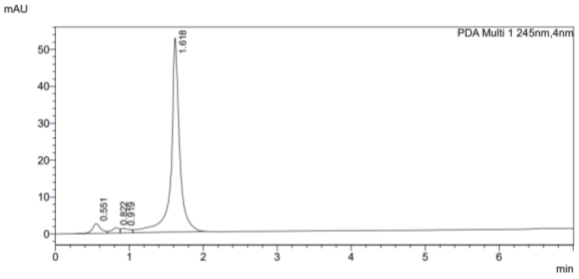

Fig. 8. Chromatogram of Sample B

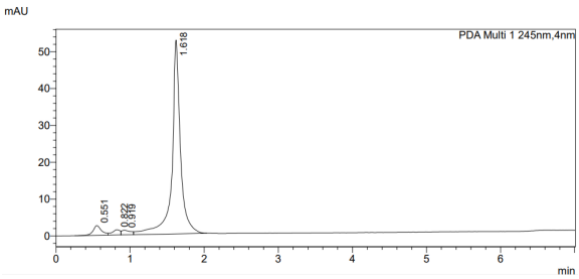

Fig. 9. Chromatogram of Sample C

4. CONCLUSION

The HPLC condition optimization results which were obtained from the optimization results using the RSM-BBD method were obtained at a percentage of methanol mobile phase in water of 34.4%, mobile phase flow rate of 1 mL/min, and column temperature of 30°C. Analysis of paracetamol content of paracetamol in jamu pegal using HPLC has met the validation parameters with test parameters including selectivity (λmax 245 nm; paracetamol retention time ± 2.6 minutes; resolution ± 2.5), linearity (r2 = 0.9984), LOD (2.44 ppm), LOQ (7.40 ppm), accuracy (97-102%), and precision (0.26-0.69%). The analysis of paracetamol content in the three samples of jamu pegal linu sold in Pasar Besar Malang City showed that negative for paracetamol and fulfilled the regulation of the Indonesian Ministry of Health Regulation No.7 in 2012.

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