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Validation of An HPLC Method For The Determination of Dexamethasone In *Jamu Pegal Linu*

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Abstract: *Pharmaceutical adulterants are still frequently detected in traditional medicines, including jamu pegal linu. Dexamethasone, a corticosteroid, is often illicitly added to enhance analgesic and anti-inflammatory effects. According to the National Agency for Drug and Food Control of the Republic Indonesia (NADFC RI) regulations, traditional medicines are prohibited from containing Active Pharmaceutical Ingredients (APIs). Although dexamethasone identification is commonly performed using Thin Layer Chromatography (TLC) and spectrophotodensitometry, High-Performance Liquid Chromatography (HPLC) offers higher resolution and improved analytical performance. This study aimed to optimize and validate an HPLC method for the determination of dexamethasone in jamu pegal linu. Method validation was performed based on linearity, specificity, accuracy, precision, limit of detection (LOD), and limit of quantification (LOQ). Mobile phase optimization was conducted using four methanol–water ratios (55:45, 60:40, 65:35, and 70:30). The optimal mobile phase was methanol–water (60:40, v/v), producing a retention time of 6.744 minutes, resolution of 2.339, tailing factor of 1.123, and theoretical plate number of 2,262. The validated method demonstrated reasonable specificity, excellent linearity (r = 0.9988), LOD of 1.147 µg/mL, LOQ of 3.823 µg/mL, 101.107% recovery, and precision (%RSD) of 1.918%.*

Keywords: *HPLC, Method Validation, Dexamethasone, Jamu Pegal Linu, Pharmaceutical Adulterants.*

INTRODUCTION

Based on reports from the National Agency for Drug and Food Control of the Republic Indonesia (NADFC RI) between 2021 and 2024, several traditional medicines were found to contain Active Pharmaceutical Ingredients (APIs). One of the most frequently detected adulterants is dexamethasone in *jamu pegal linu* products (NADFC RI, 2022; 2023; 2024). Dexamethasone is a synthetic corticosteroid that provides rapid analgesic and anti-inflammatory effects; however, uncontrolled consumption may cause serious adverse effects (Saputra, 2015). Currently, dexamethasone identification in traditional medicines is commonly performed using Thin-Layer Chromatography (TLC) and spectrophotodensitometry (NADFC RI, 2018). While TLC is simple and cost-effective, it is primarily qualitative and does not provide accurate quantification (Lintang et al., 2024). Spectrophotodensitometry offers quantification analysis but may be affected by poor sample application and elution variability (Wirasuta et al., 2014). Furthermore, dexamethasone can also be identified using high-performance liquid chromatography (HPLC). The HPLC method offers fast, efficient, and high-resolution separation (Lintang et al., 2024).

Identification of dexamethasone using the HPLC method has been carried out by Riswanto et al (2017) using a chromatography system with a mobile phase of methanol: water (65:35) with a C18 column measuring 4.6mm x 250mm, a UV detector with a wavelength of 239nm, an injection volume of 10 µL, and a flow rate of 1mL/minute. From this study, retention time of 7.56 minutes, resolution of 2.108, retention factor of 72.3, tailing factor of 1.639, and theoretical plate count of 7,719,505 were obtained. A chromatography system must be able to produce chromatograms with resolution, theoretical plates, and tailing factors that meet the requirements, and in a relatively short analysis time. To achieve optimal chromatographic separation, mobile-phase optimization is required. The non-use of the mobile phase comparison contained in the journal of Riswanto et al., 2017, was due to differences in the samples

used. In the journal, the sample tested was in powder form. Meanwhile, the sample used in this study was a *jamu pegal linu*. Differences in the composition of the ingredients used, in terms of their characteristics and properties, prompted the researchers first to optimize the mobile phase and then proceed to validation testing. This study aims to determine the optimal chromatography system for separating dexamethasone compounds and to validate the analysis method using validation parameters, namely linearity, specificity, LOD, LOQ, accuracy, and precision (Sugihartini et al., 2014).

METHODS

Materials

Dexamethasone reference standard was purchased from *Pusat Pengembangan Pengujian Obat dan Makanan Nasional* (PPPOMN). Methanol HPLC-grade was obtained from Merck. Water for Injection was obtained from PT. Ikapharmindo Putramas.

Chromatography System

The HPLC instrument used in this study was a Shimadzu i-Series LC-2050C HPLC equipped with LabSolution software, a C18 column, and a UV detector at 239 nm. The injection volume was 20 µL, and the flow rate was 1 mL/min (Riswanto et al., 2017). For the system suitability test, dexamethasone solution was injected six times consecutively into the most optimal chromatography system (Yusransyah et al., 2014).

Standard Solution Preparation

Approximately 12.5 mg of dexamethasone was accurately weighed and transferred into a 25 mL volumetric flask, then dissolved and diluted to volume with methanol to obtain the stock solution. Subsequently, 15 mL of this stock solution was pipetted into another 25 mL volumetric flask and diluted to volume with methanol. The final solution was filtered through a 0.22 µm membrane filter and transferred into an HPLC vial (Indonesian Pharmacopoeia, 2020).

Sample Solution Preparation

Approximately 1.0 g of the herbal medicine sample was weighed and transferred into a centrifuge tube. Eight milliliters of methanol were added, and the mixture was sonicated for 15 minutes to ensure complete extraction. The solution was then centrifuged at 3500 rpm for 15 minutes. The supernatant was filtered through a 0.22 µm membrane filter. An appropriate volume (1 mL) of dexamethasone was added to the filtrate for spiked solution, and the mixture was diluted to volume in a 10 mL volumetric flask with methanol. The final solution was transferred into an HPLC vial (Fajri, 2020).

Mobile Phase Optimization

The mobile phase consisted of methanol–water mixtures at 55:45, 60:40, 65:35, and 70:30 (v/v) under isocratic elution. Chromatographic parameters, including retention time, resolution (Rs), tailing factor (TF), and theoretical plate number (N), were evaluated to determine the optimal mobile phase.

Validation of HPLC Method

Specificity

Standard, sample, and blank solvent solutions were injected separately into the HPLC system under the optimized chromatographic conditions. The method was considered specific if no interfering peaks were observed at the retention time corresponding to dexamethasone (Pramudita, 2015).

Linearity

20 µL of the BPFI Dexamethasone standard solution was injected into the HPLC using the optimal chromatography system. Linearity was evaluated by injecting 20 µL of dexamethasone standard solutions at various concentrations under the optimized chromatographic conditions. A calibration curve was constructed by plotting peak area (y) versus concentration (x), and linear regression analysis was

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performed using the equation $y = a + bx$. where a is the intercept and b is the slope of the regression line. The correlation coefficient (r) was used to assess linearity (Sugihartini et al., 2014).

LOD and LOQ

Dexamethasone standard solutions at concentrations of 3, 6, 9, 15, 18, 21, and 24 µg/mL were prepared and injected (20 µL) into the HPLC system under optimized conditions. The LOD and LOQ were calculated using the formulas LOD = 3.3(SD/b) and LOQ = 10(SD/b), where SD is the standard deviation of the response based on the residual standard deviation of the calibration curve, and b is the slope of the calibration curve (Sugihartini et al., 2014).

Accuracy and Precision

The sample, spiked and standard solution were injected into the HPLC system under optimized conditions with six replicate injections. Accuracy was evaluated based on percent recovery (% recovery), while precision was assessed using the relative standard deviation (%RSD) (Susilawan et al., 2019). Percent recovery was calculated using the equation:

$$Recovery = \frac{C_f - C_A}{C^*_A} \times 100$$

where:

C_f = measured concentration of the spiked sample
C_A = concentration of the unspiked sample
C^*_A = Concentration of the added analyte.

RESULTS AND DISCUSSION

Mobile Phase Optimization

Mobile phase optimization was performed to achieve adequate chromatographic separation, symmetrical analyte peak shapes, and compliance with system suitability acceptance criteria (Sabaruddin et al., 2022). The evaluated chromatographic parameters included retention time (tR), tailing factor (TF), resolution (Rs), and theoretical plate number (N). Retention time (tR) is defined as the time interval between sample injection and the time of analyte peak maximum detection. In this study, an optimal retention time was described as one that provides sufficient separation of dexamethasone within a reasonable analysis time. The tailing factor (TF) reflects peak symmetry and directly influences quantification accuracy. According to the United States Pharmacopeia (USP, 2021), acceptable peak symmetry is indicated by a tailing factor of ≤ 2. Resolution (Rs) measures the degree of separation between two adjacent peaks. Adequate separation is achieved when Rs ≥ 1.5 (Gandjar and Rohman, 2007). The theoretical plate number (N) indicates column efficiency. A value of N ≥ 2000 is generally considered acceptable for analytical separations (USP, 2021). The optimization results obtained using four different mobile phase compositions are presented in Table 1.

The results of mobile phase optimization were analyzed using one-way ANOVA, showing statistically significant differences ($p < 0.05$) for all evaluated parameters, including retention time ($p = 0.000$), tailing factor ($p = 0.002$), resolution ($p = 0.044$), and theoretical plate number ($p = 0.000$). However, beyond statistical significance, the methanol–water ratio also influenced the chromatographic behavior of dexamethasone. Increasing the methanol content reduced the polarity of the mobile phase, thereby weakening the interaction between dexamethasone, a relatively nonpolar corticosteroid, and the nonpolar stationary phase. As a result, higher methanol proportions led to shorter retention times and, in some cases, reduced resolution due to faster elution. Conversely, lower methanol content enhanced analyte retention and improved separation, although at the expense of longer analysis time and potential peak broadening. Variations in tailing factor and theoretical plate number further reflected differences in mass transfer efficiency and analyte–stationary phase interactions under varying solvent strengths.

Based on these considerations, the selection of the optimal mobile phase was not solely determined by statistical significance but also by achieving an appropriate balance between retention,

resolution, peak symmetry, and column efficiency in accordance with system suitability requirements. As shown in Table 1, Method II (methanol–water 60:40, v/v) fulfilled all criteria (TF ≤ 2, Rs ≥ 1.5, and N ≥ 2000) and was therefore selected for further validation. Under these conditions, dexamethasone in *jamu pegal linu* was eluted at 6.74 minutes, with a tailing factor of 1.12 and a theoretical plate number of 2,262, indicating acceptable peak symmetry and efficiency.

Table 1. Chromatographic Parameters Obtained from Mobile Phase Optimization Using Different Methanol–Water Ratios

Mobile Phase (Methanol:Water)		**Parameters**			
		Retention time (minutes)	**Tailing Factor (TF ≤ 2)**	**Resolution (Rs > 1,5)**	**Theoretical Plate (N > 2000)**
Dexamethasone					
Method I (55:45)		2.760	1.350	1.850	4295
Method II (60:40)		6.792	1.097	3.537	2225
Method III (65:35)		4.684	-	0.341	1501
Method IV (70:30)		3.497	3.512	1.072	1537
Spiked					
Method I (55:45)	1	2.596	-	1.028	876
	2	2.629	-	1.084	966
	3	2.679	-	0.374	978
Method II (60:40)	1	6.763	1.125	2.520	2242
	2	6.740	1.118	2.320	2275
	3	6.729	1.125	2.179	2270
Method III (65:35)	1	4.677	-	1.031	1735
	2	4.672	-	0.935	1732
	3	4.667	-	0.974	1856
Method IV (70:30)	1	3.445	0.950	2.358	1128
	2	3.667	0.842	2.788	1132
	3	3.731	-	0.632	1135

When compared with previously reported methods, the analysis time obtained in this study falls within the typical range for RP-HPLC determination of dexamethasone. Some methods report shorter retention times (e.g., approximately 2.9 minutes) (Desnita et al., 2025), while others involving multi-analyte separation require longer run times of 7–15 minutes (Haj-Ali et al., 2025; Arif & Ata, 2020; Razzaq et al., 2017; Shahzad et al., 2023). Thus, the proposed method provides a reasonable compromise between analysis time and separation performance, particularly considering the complexity of the herbal matrix.

The theoretical plate number (N) obtained in this study ranged from 2,225 to 2,262, which is lower than values reported in previous studies, such as that of Riswanto et al. (2017), where significantly higher efficiency was observed. This discrepancy may be attributed to several factors. First, the analysis in this study was performed on a complex jamu matrix, which may contain co-extracted compounds that interfere with analyte migration and contribute to peak broadening, thereby reducing column efficiency. In contrast, previous studies often employed standard solutions or simpler matrices, which tend to produce higher N values. Second, differences in chromatographic conditions, including mobile-phase composition, flow rate, and system configuration, can significantly affect column efficiency. Despite the relatively low theoretical plate number, the system suitability parameters in this study still met the required criteria, indicating that the method remains acceptable for the intended analytical purpose.

Before method validation was performed, a system suitability test was conducted. System suitability testing is used to evaluate the performance and reliability of the chromatographic system before and during analysis (Indonesian Pharmacopoeia, 2020). The test was carried out by injecting a

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standard solution at a single concentration into the HPLC system under the selected chromatographic conditions for six consecutive replicate injections.

Table 2. System Suitability Parameters for Dexamethasone

Rep	Area	Retention time (min)
1.	13606557	6,817
2.	13479196	6,714
3.	13331860	6,686
4.	13324802	6,668
5.	13152706	6,660
6.	13023509	6,665
%RSD	**1.59%**	**0.89%**

Based on the results of six replicate injections of the dexamethasone (Table 2), the %RSD of the peak area was 1.587% and 0.89% for retention time. This value complies with the acceptance criterion of less than 2% as specified in the *Indonesian Pharmacopoeia* (6th ed.). Since the obtained %RSD value was below the required limit, the chromatographic system demonstrated acceptable repeatability and stability. Therefore, the HPLC system was considered suitable for further method validation (Aziz, 2020).

Specificity

Specificity testing was performed to evaluate the ability of the method to unequivocally assess the analyte in the presence of potential interferences, including impurities and matrix components. The chromatographic peak of the analyte should be free from co-eluting substances and demonstrate adequate separation from other components in the sample matrix (Ayuningtyas, 2021). The specificity results are presented in Figure 1. Dexamethasone in the spiked sample was well separated at a retention time of approximately 6.7 minutes, with no interfering peaks observed at or near the retention time of the analyte. These results indicate that the developed HPLC method is specific and capable of effectively separating dexamethasone from other components in the sample matrix.

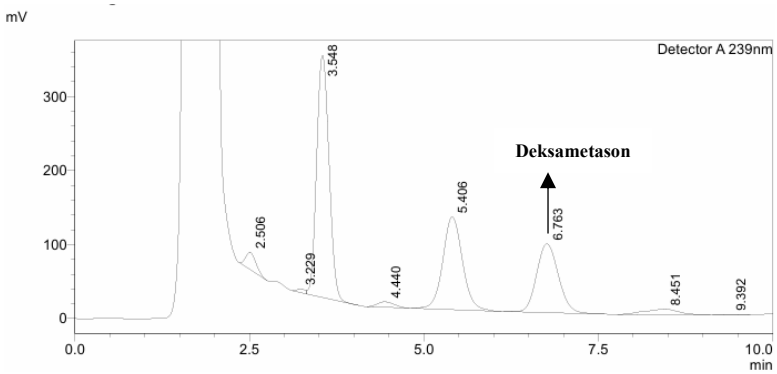

Figure 1. Chromatogram of the spiked sample for specificity evaluation.

Linearity

Linearity was evaluated to determine the relationship between analyte concentration and instrument response over a specified concentration range. The correlation coefficient (r) was used to assess the strength of the linear relationship between concentration and response. The linearity results are presented in Figure 2. The calibration curve was constructed using seven concentrations of the dexamethasone reference standard (BPFI). The obtained correlation coefficient (r = 0.9988) meets the

AOAC (2002) acceptance criterion ($r \geq 0.990$), indicating excellent linearity over the tested concentration range.

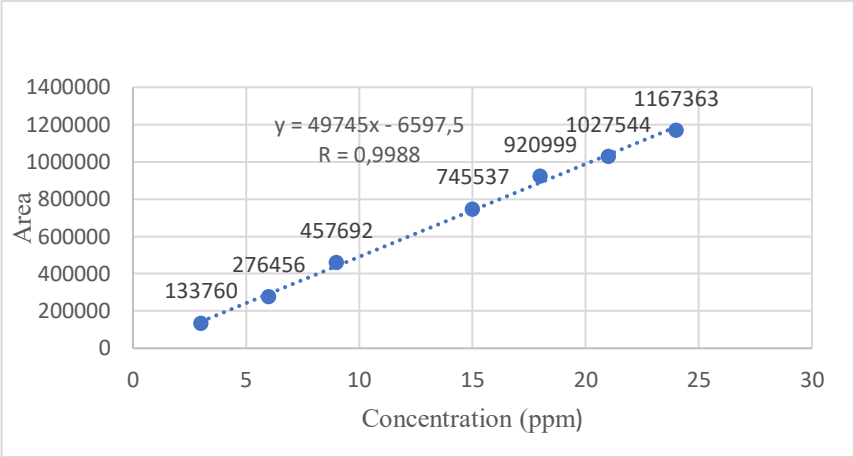

Figure 2. Calibration curve of dexamethasone.

LOD and LOQ

The limit of detection (LOD) and limit of quantification (LOQ) represent the lowest concentrations of analyte that can be detected and quantitatively determined with acceptable accuracy and precision, respectively (AOAC, 2002). Based on the results presented in Table 3, the LOD and LOQ for dexamethasone were 1.147 µg/mL and 3.823 µg/mL, indicating that the method is capable of detecting and reliably quantifying low levels of analyte under the established experimental conditions.

Table 3. LOD and LOQ values for dexamethasone.

Concentration (X)	**Area (Y)**	**Y'**	**Y-Y'**	**$(Y-Y')^2$**
3ppm	133760	142637,5	-8877,5	78810006,25
6ppm	276456	291873	-15416,5	237668472,3
9ppm	457692	441108	16584,5	275045640,3
15ppm	745537	739578	5959,5	35515640,25
18ppm	920999	888813	32186,5	1035970782
21ppm	1027544	1038048	-10503,5	1103223512,3
24ppm	1167363	1187283	-19919,5	396786480,3
Y = 49745x - 6597,5 R= 0,9988				**$\sum$= 2170120534**
Slope (b) = 49745				
Standard Deviation (SD) = 19018,1				
LOD			**LOQ**	
$LOD = 3{,}3\frac{SD}{S}$			$LOQ = 10\frac{SD}{S}$	
$LOD = 3{,}3\frac{19018{,}1}{49745}$			$LOQ = 10\frac{19018{,}1}{49745}$	
LOD = 1,14693			LOQ = 3,82311	
LOD = 1,147 µg/mL			LOQ = 3,8231 µg/mL	

The LOD (1.15 µg/mL) and LOQ (3.82 µg/mL) obtained for jamu are comparable to those reported for dexamethasone sodium phosphate in nasal microspheres (LOD 1.33 µg/mL, LOQ 4.43 µg/mL) (Desnita et al., 2025), although less sensitive than methods optimized for biological fluids, which reach ng/mL levels due to more advanced detection systems and extensive sample preparation (Razzaq et al., 2017; Shahzad et al., 2023). Furthermore, the method demonstrated good accuracy and

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precision, with a recovery of approximately 101% and %RSD of 1.9%. These values are consistent with, or slightly better than, those reported in previous studies involving dexamethasone in pharmaceutical and herbal matrices, which generally show recoveries in the range of 95–103% and %RSD below 2–3% (Riswanto et al., 2017; Haj-Ali et al., 2025; Arif & Ata, 2020; Shahzad et al., 2023). These comparisons indicate that the validated method offers adequate sensitivity and robust performance for routine screening of dexamethasone adulteration in *jamu pegal linu* as complex herbal preparations.

Accuracy and Precision

Accuracy refers to the closeness of agreement between the measured value and the actual or accepted reference value. It is commonly expressed as percent recovery (% recovery) and determined by analyzing spiked samples during sample preparation. Precision refers to the degree of agreement among a series of measurements obtained under specified conditions. It is evaluated using the relative standard deviation (%RSD) of replicate measurements (AOAC, 2002). In this study, accuracy and precision were assessed by analyzing a single concentration level of the spiked sample with six replicate injections using the selected HPLC system.

Table 4. Accuracy and Precision Results of the HPLC Method for Dexamethasone

Replication	Sample Area	Cons. Sample	*Area Spiked*	*Cons. Spiked*	Accuracy (C_f-C_A/C^*_A)x100
1.	412496	8,425	1403813	28,353	99,640
2.	350755	7,184	1341468	27,100	93,579
3.	339239	6,952	1361057	27,493	102,579
4.	394168	8,056	1381018	27,895	99,191
5.	438296	8,943	1461106	29,505	102,804
6.	356444	7,298	1378446	27,843	102,724

The results of the accuracy and precision tests are presented in Table 4. The mean percent recovery obtained was 101.107%, indicating good agreement between the measured and nominal concentrations. According to AOAC (2002), for analyte concentrations at the ppm level (20 ppm), the acceptable recovery range is 80–115%. Therefore, the obtained recovery value meets the established accuracy criteria.

The precision results showed a %RSD value of 1.918% for the spiked sample. Based on AOAC (2002) guidelines, for analyte concentrations of approximately 20 ppm, the acceptable repeatability (%RSD) is ≤ 6%. Since the obtained %RSD value is well below this limit, the method meets the precision acceptance criteria.

CONCLUSIONS

Based on the results of the HPLC method validation test for the identification of dexamethasone in *jamu pegal linu*, method II, with a mobile phase ratio of methanol: water (60:40), optimally separates the dexamethasone. The results of the HPLC method validation that meet the requirements are reasonable specificity, linearity (r = 0.9988), LOD 1.147 µg/mL, LOQ 3,8231 µg/mL, accuracy 101.107% and precision 1.918%, therefore the method can be used to determine the levels of dexamethasone in *jamu pegal linu*.

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