

Research Article

Simple Analytical Method on the Determination of Dexamethasone in Herbal Medicine

Rimadani Pratiwi , Adira Rahmawaty, and Aliya Nur Hasanah 

Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Bandung 45363, Indonesia

Correspondence should be addressed to Rimadani Pratiwi; rimadani.pratiwi@unpad.ac.id

Received 30 June 2022; Revised 2 August 2022; Accepted 5 September 2022; Published 27 September 2022

Academic Editor: K. S. V. Krishna Rao

Copyright © 2022 Rimadani Pratiwi et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Chemical drugs have been added to some of the traditional herbal medicine to enhance the therapeutic effect. One of them is dexamethasone which is added in herbal pain relief. To determine the content of dexamethasone in herbal medicine, a quantitative analytical method was developed with the reaction of the formation of a dexamethasone-hydroxylamine complex with fluoranil which was measured by UV-Vis spectrophotometry. Under optimized conditions, the performance of this system to analyze dexamethasone exhibited a good analytical response. The correlation coefficient was (R^2) of 0.9965 from a concentration of 1–40 g/mL, LOD, and LOQ of 0.21 $\mu\text{g/mL}$ and 0.64 $\mu\text{g/mL}$, % recovery for concentrations of 80%, 100%, and 120%, are 100.14% \pm 0.58; 98.35% \pm 1.19; and 99.00% \pm 1.18, respectively; and %RSD is 0.75%. The sixteen herbal medicine samples were analyzed to confirm the application of this method. The result showed six samples confirmed containing dexamethasone with levels ranging from 7.44 to 21.61 $\mu\text{g/mL}$ and confirmed with HPLC data. The UV-Vis spectrophotometry method is simple and effective for dexamethasone analysis in herbal medicine.

1. Introduction

Herbal medicine is still widely used as a drug or an alternative medicine to treat diseases. Herbal medicines are naturally occurring plant-derived substances used for treatment or medicinal purposes [1]. To their definition, herbal medicine should not contain a synthetic chemical or medicinal isolation result. Since the market for herbal medicine is growing up continuously, there are reports that herbal medicine contains an undeclared synthetic drug to increase the therapeutic effect [2]. One of the undeclared synthetic drugs in herbal medicine is dexamethasone [3, 4].

Dexamethasone is a glucocorticoid that acts as a very potent and long-acting anti-inflammatory agent with the mechanism of action of reducing the production of inflammatory mediators such as suppressing the increase in neutrophils, reversing the increase in capillary permeability, and suppressing the immune response [5–7]. However,

uncontrolled consumption of dexamethasone will cause some adverse effects such as vomiting, malaise, insomnia, anxiety until psychosis, aggravation of schizophrenia [6], and moon face [8]. Therefore, monitoring dexamethasone in herbal medicine is important.

Several analytical methods have been used for detecting dexamethasone in herbal medicine, including high-performance liquid chromatography [9], infrared spectroscopy that combined with the partial least square (PLS) [10], thin layer chromatography (TLC)—densitometry [11], and paper-based analytical device [4]. The development of dexamethasone analysis methods is continuing to find the alternative method that is low cost, effective, and sensitive. One of them is by using a spectrophotometric approach. Al-Janabi et al. developed a colorimetric determination of dexamethasone in tablets using UV-Vis spectrophotometry [12]. They used fluoranyl as a reagent to detect dexamethasone-hydroxylamine complex, and it was measured by using UV-Vis spectrophotometry.

In this research, we measured dexamethasone in herbal medicine using UV-Vis spectrophotometry. First, we extracted dexamethasone using chloroform: methanol (9:1, v/v), continued with complexation of dexamethasone with hydroxylamine, and then made to react with fluoranyl. The result shows that this method fulfilled the requirement of method validation and shows a good agreement result compared with high-performance liquid chromatography (HPLC) method.

2. Materials and Methods

2.1. Materials and Instruments. All chemicals used were of analytical grade and used without further purification. Propionic acid, chloroform, sodium acetate, and methanol were purchased from Merck. Dexamethasone was obtained from PT. Kimia Farma. Ethanol was purchased from ACS chemical and fluoranyl from Fisher. Hydroxylamine hydrochloride was purchased from Lobo Chemie. Methanol for HPLC was purchased from Merck. The absorbance measurement was recorded by UV-visible spectrophotometer Analytik Jena Specord 200 using a 1.0 cm quartz cell. High-performance liquid chromatography (HPLC) used from Shimadzu series SPD-10Avp, UV Detector.

2.2. Collecting and Preparation of Herbal Medicine Samples. The samples were collected from pharmacies, traditional drug stores, and traditional markets circulating in Ciawi District, Bogor Regency, that claimed efficacy in relieving pain, aches, and gout. The preparation steps are a modified method from Khoirunnisa's research with the addition of the centrifuge step after mixing the sample with the solvent. Each sample was weighed at 6 g and then dissolved in 30 mL of a mixture of chloroform: methanol (9:1, v/v) [13]. This solution was then shaken for 30 minutes and centrifuged for 5 minutes at 4000 rpm [4]. The filtrate was taken and evaporated over a water bath at a temperature of 70°C, the remaining evaporation was dissolved in 5 ml of methanol [13]. This preparation performed well on blanks, negative control, and positive control.

2.3. Screening Sample with Thin Layer Chromatography (TLC). The prepared herbal medicine samples were spotted on a TLC plate (GF silica gel 254 nm) with a size of 8 × 10 cm and the spotting distance between samples was 1.5 cm. The mobile phase is used based on Clarke's which is chloroform: methanol: propionic acid with a ratio of 72:18:10 [14]. The results are observed under a UV light of 254 nm.

2.4. Optimization of Optimum Conditions and Time for the Formation of Dexamethasone-Hydroxylamine Complexes. A 20 mg of standard dexamethasone was mixed with variations of hydroxylamine hydrochloride (10 mg, 20 mg, 30 mg) and 20 mg of sodium acetate. Then dissolved in 25 mL of ethanol and refluxed at varying times for 30 minutes, 60 minutes, and 120 minutes. After refluxing, the ethanol contained in the solution was evaporated at a

temperature of 70°C. The residue formed was extracted with 10 mL of chloroform and 10 mL of water. The organic phase (chloroform) was transferred into a 50 mL volumetric flask. The complex formed was measured using UV-Vis spectrophotometry with a wavelength range of 200–400 nm to find the maximum absorbance. Blanks and positive controls were also run with the same procedure.

2.5. Performance of UV-Vis Spectrophotometry. A 100 ppm standard solution of dexamethasone dissolved in chloroform into various concentrations of 1, 5, 10, 20, 30, and 40 ppm was then measured using UV-Vis spectrophotometry at a wavelength of 241 nm. Linearity, the limit of detection (LOD), and quantification (LOQ) were determined to measure the performance of the method. The precision of this method was determined by measuring 6 standard solutions of dexamethasone in the same concentration (20 g/mL) using UV-Vis spectrophotometry at a wavelength of 241 nm. The accuracy was performed by spiking the sample solution with standard dexamethasone in 3 different concentrations (80%, 100%, 120%).

2.6. Analysis of Dexamethasone in Herbal Medicine Sample. A 20 mg of herbal medicine sample, 20 mg of hydroxylamine hydrochloride, and 20 mg of sodium acetate were mixed and dissolved in 25 mL of ethanol, and then refluxed for 60 minutes. After refluxing, the ethanol contained in the solution was evaporated at a temperature of 70°C. The residue formed was extracted with 10 mL of chloroform and 10 mL of water. The organic phase (chloroform) was transferred into a 50 mL volumetric flask. The complex of dexamethasone-hydroxylamine is formed. Aliquots of dexamethasone-hydroxylamine solution were reacted with fluoranyl reagent (1:1). The absorbance of the colored solution was measured using UV-Vis at a wavelength of 483 nm. Blanks and positive controls were also run with the same procedure.

To ensure the result of the UV-Vis method, the sample was also determined using the high-performance liquid chromatography (HPLC) by reference to the Indonesian pharmacopeia with some adjustments to the mobile phase and flow rate. The standard solution and the sample solution were prepared by dissolving them, respectively, in methanol. Then, separately inject each equal volume of 20 µL of standard solution and sample solution into the autosampler, record the chromatogram, and measure the peak response of the standard solution and sample solution [15]. The mobile phase used was a mixture of acetonitrile: water (7:3) [16] with a flow rate of 0.8 mL per minute, and a running time of 5 minutes.

3. Results and Discussion

3.1. Screening Sample with Thin Layer Chromatography (TLC). This step was performed to screen the samples that contain dexamethasone. From 16 samples of herbal medicine that had been collected and prepared, it was found that 6 samples of herbal medicine were detected containing

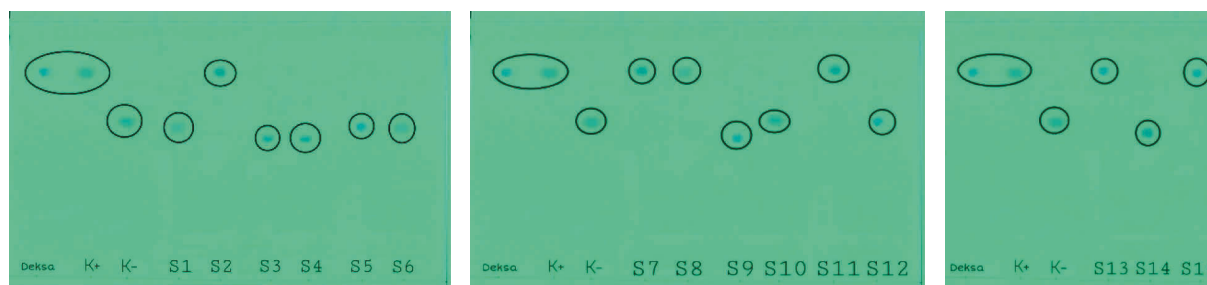


FIGURE 1: Result of screening herbal medicine with TLC compared with standard, control positive (K+), and control negative (K-).

dexamethasone after comparing the results of the RF values obtained in TLC with positive and negative controls (Figure 1). Based on this, the 6 samples of herbal medicine were continued for analysis using UV-Vis spectrophotometry and HPLC for confirmation.

3.2. Optimization of Optimum Conditions and Time for the Formation of Dexamethasone-Hydroxylamine Complexes.

Optimization of the conditions and time of formation of the dexamethasone-hydroxylamine complex aims to ensure and compare the best condition and time so the complex can be formed maximally by looking at the results of the percent recovery from the variations tested. The results of the percent recovery in Table 1 showed that the best conditions were when the addition of hydroxylamine hydrochloride was 20 mg and the best reflux time was 60 minutes. It is because of the less reflux time used, the complex reaction between dexamethasone and hydroxylamine has not been maximally formed and there are still residual substances that have not reacted, but on the other hand, if the reflux time is too long, the complex reaction that has been formed will slowly be degraded [12, 17].

3.3. Performance of UV-Vis Spectrophotometry.

Performance of UV-Vis spectrophotometry has been carried out by validation to prove that the method has met the requirements of several parameters [18]. The determination of the wavelength of dexamethasone was carried out by measuring the standard dexamethasone as much as triple times in the wavelength range of 200–400 nm. The measurement results shows the maximum readable dexamethasone at a wavelength of 241 nm. This is following the literature which shows that dexamethasone has a maximum wavelength of 241 nm [19].

The value of R^2 on linearity shows a value of 0.9965. It equipped the requirements based on Indonesian pharmacopeia, that is, $R^2 \geq 0.98$ [15]. The LOD value is 0.21 g/mL and the LOQ is 0.64 g/mL. The results were compared with another method in the determination of dexamethasone in herbal medicine [10, 16, 20], indicating that this method can detect and measure dexamethasone more sensitively because the LOD and LOQ gains are better.

The precision performed consisted of intraday precision with a %RSD value of 0.75% and an interday precision test carried out for 3 consecutive days showing the %RSD of

TABLE 1: Optimization of conditions and time for the formation of the dexamethasone-hydroxylamine complex.

Time for reflux	Variations of hydroxylamine hydrochloride		
	10 mg	20 mg	30 mg
30 min	44.06% \pm 1.10	82.18% \pm 1.70	73.27% \pm 2.1
60 min	46.47% \pm 2.37	98.40% \pm 0.55	78.03% \pm 2.27
120 min	54.95% \pm 1.01	92.80% \pm 1.18	81.93% \pm 0.95

0.78%; 0.73%; and 0.88%. It complies with the requirements based on the USP that a good value of %RSD is <2% [21]. The accuracy results showed that the results range for 80% concentration is 100.14% \pm 0.58, 100% concentration is 98.35% \pm 1.19, and for 120% concentration is 99.00% \pm 1.18. It complies with the requirements based on the Indonesia pharmacopeia that a good value of %recovery is between 98 and 102% [15].

3.4. Analysis of Dexamethasone in Herbal Medicine Sample.

Analysis of dexamethasone in herbal medicine samples was carried out by UV-Vis spectrophotometry as the research method and HPLC as the standard method. The reaction that occurs between the dexamethasone-hydroxylamine complex and fluoranyl indicates that the carbonyl group of dexamethasone which has been modified into a dexamethasone-hydroxylamine complex will contain a weak acid hydroxyl group so that it easily reacts with fluoranyl which then causes the formation of a complex color that can be detected by UV-Vis spectrophotometry [12]. It happens because fluoranyl is a strong π (phi) acceptor. The acceptor is a collection of ligands containing bonds. This bond can accept electrons from metal orbitals if they have comparable energy but higher electron affinity than the donor ligand [22].

This complex was measured as much as triple times in the wavelength range of 400–800 nm. It is because the solution of the complex has visible color which can be detected with this wavelength [23]. The measurement results show the maximum readable dexamethasone at a wavelength of 483 nm. This is close to the following literature which shows that the reaction of dexamethasone-hydroxylamine complex and fluoranyl has a maximum wavelength of 485 nm [12]. The deviation can still be tolerated according to the European pharmacopeia which is ± 3 nm for visible light [24].

To demonstrate the applicability of this research method for real sample analysis, 6 positive samples containing

TABLE 2: Comparison of analysis of herbal samples with UV-Vis spectrophotometry and HPLC ($n=3$).

Samples	Concentration		Deviation
	UV-Vis spectrophotometry	HPLC	
Standard	19.88 ± 0.42	19.88 ± 0.49	0.00 ± 0.07
Positive control	31.03 ± 0.81	31.07 ± 0.83	0.04 ± 0.02
Sample 2	7.44 ± 0.02	7.45 ± 0.02	0.01 ± 0.00
Sample 7	12.23 ± 0.31	12.48 ± 0.38	0.25 ± 0.07
Sample 8	11.11 ± 0.58	11.15 ± 0.52	0.04 ± 0.06
Sample 11	16.65 ± 0.18	16.76 ± 0.15	0.11 ± 0.03
Sample 13	5.68 ± 0.10	5.72 ± 0.04	0.04 ± 0.06
Sample 15	21.61 ± 0.40	21.62 ± 0.37	0.01 ± 0.03

dexamethasone were analyzed using UV-Vis spectrophotometry as the research method and HPLC as a standard method. The results are summarized in Table 2 showing good agreement between UV-Vis spectrophotometry and HPLC. This suggests that the analytical method using UV-Vis spectrophotometry with the reaction of the formation of dexamethasone-hydroxylamine complexes reacted with fluoranyl can be used as an analytical method to detect the drug chemical dexamethasone in herbal medicine samples.

4. Conclusion

The optimum conditions, for the determination of dexamethasone in herbal medicine based on the reaction of forming a dexamethasone-hydroxylamine complex with fluoranyl, is the addition of 20 mg of hydroxylamine hydrochloride with the optimum reflux time is 60 minutes. The validation method showed good results and met the requirements with the LOD and LOQ values of 0.21 g/mL and 0.64 g/mL, respectively. This simple developed method was also successful in the determination of dexamethasone in herbal medicine samples which showed a good agreement with the HPLC method.

Data Availability

All data are available in this manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This research was funded by the Directorate of Research and Community Engagement Universitas Padjadjaran through Internal Research Grant 2022 No. 2203/UN6.3.1/PT.00/2022.

References

- [1] O. Woerdenbag and H. J. Kayser, "Indonesian traditional herbal medicine towards rational phytopharmacological use," *Journal of Herbal Medicine*, vol. 4, pp. 51–73, 2014.
- [2] R. Pratiwi, R. H. F. Dipadharma, I. J. Prayugo, and A. L. Layandro, "Recent analytical method for detection of chemical adulterants in herbal medicine," *Molecules*, vol. 26, 2021.
- [3] Badan POM, *Lindungi Masyarakat dari Obat Tradisional, Suplemen Kesehatan, Dan Kosmetik Yang Berisiko Terhadap Kesehatan*, Badan POM, Jakarta, Indonesia, 2020.
- [4] V. Primpray, O. Chailapakul, M. Tokeshi, T. Rojanarata, and W. Laiwattanapaisal, "A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines," *Analytica Chimica Acta*, vol. 1078, pp. 16–23, 2019.
- [5] ASHP, *Dexamethasone: Medline Plus Drug Information*, American Society of Health-System Pharmacists, Bethesda, MD, USA, 2021.
- [6] R. J. Flower and F. Gavins, "Dexamethasone," *xPharm: The Comprehensive Pharmacology Reference*, pp. 1–6, 2008.
- [7] MIMS, "Dexamethasone," 2021, <https://www.mims.com/indonesia/drug/info/dexamethasone?mtype=generic>.
- [8] K. A. Cendekiawan, S. Winarso, and A. C. Novi Marchianti, "Surveilans penyalahgunaan bahan kimia sintesis deksametason pada jamu pegal linu menggunakan metode near infra red dan kemometri," *A Multidisciplinary Journal*, vol. 2, no. 1, p. 30, 2019.
- [9] N. Klinsunthorn, A. Petsom, and T. Nhujak, "Determination of steroids adulterated in liquid herbal medicines using QuEChERS sample preparation and high-performance liquid chromatography," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 55, no. 5, pp. 1175–1178, 2011.
- [10] A. Nugroho and F. D. Ritonga, "Rapid analysis of adulterated dexamethasone in joint-pain killer traditional herbal medicine (THM) using infrared spectroscopy," *EKSAKTA: Journal of Sciences and Data Analysis*, vol. 18, no. 2, pp. 137–145, 2018.
- [11] D. A. I. Permatasari, N. Kurniasri, M. P. Mahardika, and M. P. Mahardika, "Qualitative and quantitative analysis of dexamethasone in rheumatic pain herbal medicine using Thin layer chromatography (TLC)—densitometry," *Journal of Fundamental and Applied Pharmaceutical Science*, vol. 2, no. 1, pp. 10–22, 2021.
- [12] K. W. S. Al-Janabi, A. K. Mahmood, and H. M. Luaibi, "Development of a simple colorimetric determination of dexamethasone," *International Journal of Drug Delivery Technology*, vol. 10, no. 2, pp. 255–258, 2020.
- [13] S. M. Khoirunnisa, "Identifikasi deksametason dalam jamu pegal linu sediaan serbuk yang beredar di Pasar-pasar kota bandar lampung secara kromatografi lapis tipis," *Journal of Science and Application Technology*, vol. 2, no. 1, pp. 94–101, 2017.
- [14] A. C. Moffat, M. . & David, and W. Brian, *Clarke's Analysis of Drug's and Poisons*, Pharmaceutical Press, London, UK, 2011.
- [15] R. I. Kemenkes, *Farmakope Indonesia Edisi VI*, Kementerian Kesehatan RI, Jakarta, Indonesia, 2020.
- [16] A. D. Ananto, U. Y. M. G. Lalu, and S. W. F. A. Lalu, "Analysis of BKO content (antalgin and dexamethasone) in herbal medicine using iodimetry titration and HPLC method," *Elkawnie*, vol. 6, no. 1, 2020.
- [17] S. P. Levine, T. M. Harvey, T. J. Waeghe, and R. H. Shapiro, "O-alkyloxime derivatives for gas chromatographic and gas chromatographic-mass spectrometric determination of aldehydes," *Analytical Chemistry*, vol. 53, no. 6, pp. 805–809, 1981.
- [18] Ema, "ICH harmonised tripartite guideline validation of analytical procedures: text and methodology Q2(R1)," in

Proceedings of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, Geneva, Switzerland, 2005.

- [19] K. Anand and A. Pandey, "Validation study of steroidal drugs (dexamethasone and betamethasone) by U.V. spectrophotometric method," *Asian Journal of Pharmaceutical and Clinical Research*, vol. 11, no. 7, pp. 501–505, 2018.
- [20] N. S. Kim, J. Kim, N. Y. Lim, J. H. Lee, S. Park, and H. Kang, "Simultaneous determination of illegal drug substances in dietary supplements for gout and osteoporosis using ultra-performance liquid chromatography and liquid chromatography-quadrupole-time-of-flight mass spectrometry," *Journal of Pharmaceutical and Biomedical Analysis*, vol. 179, Article ID 113003, 2020.
- [21] USP, *United States Pharmacopeia Edisi 36*, United States Pharmacopeia, Rockville, MD, USA, 2011.
- [22] A. Bakhshi, R. . Verma, I. Kumar, and D. Gupta, "Inorganic chemistry-III (metal complexes and metal clusters)," 2021, https://epgp.inflibnet.ac.in/epgpdata/uploads/epgp_content/chemistry/inorganic_chemistry-iii/01.acidity,_metal_carbonyls,_their_classification_and_general_features/et/4829_et_et.pdf.
- [23] W. Suarsa, "Spektroskopi," 2015, https://simdos.unud.ac.id/uploads/file_pendidikan_dir/610b308c39ca975868e39e01ec9e9ed5.pdf.
- [24] Council of Europe, *European Pharmacopoeia*, Council of Europe, Strasbourg, France, 2014.