

Development and validation of a HPLC method for the simultaneous determination of chlorquinaldol and promestriene in complex prescription

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복방제제 내 클로르퀴날돌과 프로메스트리엔에 대한 HPLC 기반 동시분석법의 개발 및 밸리데이션

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Abstract: Currently, many types of compound preparations are being used but the quality control guidelines for their use are lacking. In case of single compound drug, the quality control methods are specified in the pharmacopeia. However, there is no method to simultaneously analyze compound preparations. In this study, a simple validated analytical method for HPLC separation of chlorquinaldol and promestriene is introduced. Validation was divided into categories including linearity, precision, accuracy (recovery) and system suitability. The contents of the products which are on the market were monitored using the validated analytical method and the robustness of the analytical method was tested by conducting an inter-laboratory validation.

요 약: 현재 시중에 많은 복방제제가 유통되고 있음에도 불구하고 이의 품질관리를 위한 시험법은 부족한 실정이다. 여러 주효 성분을 가지는 복방제제의 경우 대부분 약전에 수재된 항목 중 2개 이상의 조합으로 구성되어 있으나 약전에 단일 항목에 대한 시험법이 고시되어 있을 뿐 여러 항목을 한번에 시험하는 방법이 없어 한가지 제제를 관리 하는 데에 여러 시험법이 요구되어 효율이 낮고 비용이 높다. 따라서 본 연구는 현재 유통중인 복방제제의 새로운 분석법을 개발한 과정과 그 때에 적용되는 기준을 제시하고 그 결과를 검증하였다. 복방 프로메스트리엔, 클로르퀴날돌 제제에 대한 새로운 분석법을 HPLC

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를 기반으로 가장 적용이 용이하게 개발하였으며 직선성, 정밀성, 정확성(회수율), 시스템 적합성(반복성, 분리도) 등의 항목에 대하여 밸리데이션을 수행함으로써 분석법을 검증하였다. 또한 개발된 시험법을 이용하여 시중 제품을 모니터링하고 실험실간 밸리데이션을 수행하여 시험법의 견고성을 확인함으로써 새로운 고시안으로서의 가능성을 제시하였다.

Key words: Quality control guidelines, Validation, High performance liquid chromatography (HPLC), Chlorquinaldol, Promestriene

1. Introduction

Chlorquinaldol (5,7-dichloro-2-methylquinolin-8-ol) is a local anti-infective agent used for skin, gastrointestinal and vaginal infections against fungi, protozoa and certain bacteria¹⁻³ (Fig. 1a). Promestriene [(8R, 9S,13S,14S)-17-methoxy-13-methyl-3-propoxy-6,7,8,9, 11,12,14,15,16,17-decahydrocyclopenta[a] phenanthrene] is a topical synthetic version of the hormone estradiol indicated for treating various symptoms such as vaginal atrophy, vaginitis and stress urinary incontinence⁴⁻⁶ (Fig. 1b). At present, the two compounds are prescribed together to relieve vaginal atrophy and vaginitis caused by the lack of estrogen and even leucorrhea; side effects include an external genital burning sensation, itching and dryness. However, since these two compounds are used together, it is required to develop a new analytical method likely to identify and quantify the two compounds simultaneously. The analytical method notified in the Pharmacopeia takes a great deal of time and has a low efficiency since it identifies and quantifies each compound separately. As for the existing analytical method, promestriene is identified by ultraviolet-visible spectrophotometry and quantified by comparing absorbance of sample to the standard. On the other hand, chlorquinaldol is identified by using Loss-on-Ignition test and quantified by using

non-aqueous titrations. Since two different analytical methods are needed to monitor contents of one tablet, this process is quite inconvenient and inefficient. The Korean Food and Drug Administration (KFDA) is developing and improving the existing quality control guidelines for compound preparations that are currently being used. This study aims to develop a new analytical method that is applicable to the present commercial products on the market immediately.

A new analytical method should be rigid, efficient, simple and inexpensive. Further, others can apply it, likely without the need for additional equipment. Accordingly, we have developed a new analytical method based on HPLC, which is one of the most commonly used instruments in the analytical laboratory.⁷⁻¹⁹ In addition, this new method has the advantage of analyzing two drugs simultaneously and even carrying out identification and quantification at a time. For the stationary phase, a 150 mm-long, ODS reverse-phase column was used. When a new analytical method is developed on the basis of a 150 mm-long column, it has the advantage of being applied to all the columns over 150 mm in length. For the mobile phase, methanol was used rather than acetonitrile because it is inexpensive and widely used, and by attempting to separate it under the isocratic condition as much as possible, we were able to have quality control conducted easily and rapidly at the actual worksite. To analyze the two compounds simultaneously, a UV absorption wavelength was selected and compounds showed stable absorption.

To validate the new analytical method, we referred to the validation guideline protocol of the KFDA.²⁰⁻²¹ The items consist of linearity, precision, accuracy (recovery) and system suitability. Further, system

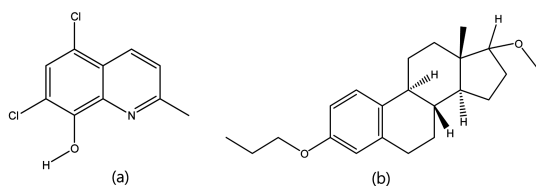


Fig. 1. Chemical structures of (a) chlorquinaldol and (b) promestriene.

suitability was composed of repeatability and resolution. We confirmed the robustness of this new analytical method by monitoring the contents of existing commercial products and carrying out inter-laboratory validation in another laboratory. This study suggests a new quality control guideline for analyzing chlorquinaldol and promestriene and it is expected that the results of this study will be used to develop a protocol for an analytical method for these two drugs.

2. Experimental

2.1. Reagents

Chlorquinaldol reference standard was purchased from Sigma-Aldrich (St. Louis, MO, USA.) Promestriene reference standard was provided from Samil Pharmaceutical Co., Ltd (Seoul, Korea). Methanol was high-purity HPLC grade from Duksan Inc., (Ansan, Korea). Distilled water was used after purification through a 0.45 μm nylon membrane filter (Whatman International Ltd, Maidstone, Kent, UK). All other reagents used were of high purity or HPLC grade. Commercially available drug containing 200 mg of chlorquinaldol and 10 mg of promestriene was provided by Samil Pharmaceutical Co., Ltd.

2.2. Standard solutions

Five solutions of different concentrations of drugs were prepared by dissolving chlorquinaldol and promestriene standard in methanol: in $\mu\text{g/mL}$ or ppm: chlorquinaldol 160 + promestriene 8, chlorquinaldol 180 + promestriene 9, chlorquinaldol 200 + promestriene 10, chlorquinaldol 220 + promestriene 11 and chlorquinaldol 240 + promestriene 12, representing 80, 90, 100, 110 and 120% of the reference concentration.

2.3. Samples preparation

20 tablets, where 1 tablet is equivalent to 200 mg chlorquinaldol and promestriene 10 mg, were weighed and finely powdered. Of them, 1/20 was dissolved in 100 mL methanol and sonicated for 30 min. This stock solution was diluted 1:15 "solution (A)," which

accounts for 80% of the standard solution. In addition, chlorquinaldol and promestriene standard were dissolved in methanol to make "solution (B)" which accounts for 160% of the standard solution. Solutions (A) and (B) were mixed in the following volume ratios to make the final sample - 100% [(A):(B) = 3:1], 110% [(A):(B) = 5:3], 120% [(A):(B) = 1:1], sonicated for 30 min and filtered using a 0.45 μm filter (Toyo Roshi Kaisha, Ltd., Tokyo, Japan) before injection into the HPLC.

2.4. Instrumentation and chromatographic condition

The HPLC system consisted of a Waters 510 pump, Waters 717 plus autosampler and Waters 486 UV detector. A Phenomenex Synergi (C18, 150 \times 4.6 mm, 4 μm) column was utilized for separating compounds, keeping the temperature at 30 $^{\circ}\text{C}$. An isocratic mobile phase system was used where the mobile phase A (water) to mobile phase B (methanol) ratio was 5:95; the flow rate was 1.0 mL/min. Sample (10 μL) was injected and samples were detected at 220 nm.

2.5. Validation

Validation was conducted according to the Korean Pharmacopeia proposed by KFDA. The categories consist of linearity, precision, accuracy (recovery) and system suitability and for the system suitability, repeatability and resolution were considered. Using the validated analytical method, the contents of the products on the market were monitored and an inter-laboratory validation was implemented which tested the robustness of the developed analytical method.

3. Results and Discussion

The objective of this study was to amend the current assay for chlorquinaldol/promestriene tablet. The analytical methods for chlorquinaldol/promestriene tablet currently listed are divided into identification and quantification and require two assays.²⁰ This takes more time and cost than needed. Thus, more practical and reliable analytical method is needed

Table 1. Results of linearity test. Slope, y intercept and r^2 of calibration curves

Chlorquinaldol				Promestriene			
Trial	Slope	y intercept	r^2	Trial	Slope	y intercept	r^2
1	4.87×10^4	-9.97×10^4	9.993×10^{-1}	1	1.00×10^3	-3.22×10^3	9.992×10^{-1}
2	4.81×10^4	-19.4×10^4	9.994×10^{-1}	2	9.89×10^2	-2.40×10^3	9.996×10^{-1}
3	4.66×10^4	1.21×10^4	9.992×10^{-1}	3	1.01×10^3	-3.00×10^3	9.999×10^{-1}
Average	4.78×10^4	-3.57×10^4	9.993×10^{-1}	Average	1.00×10^3	-2.87×10^3	9.996×10^{-1}
STDEV ^a	1.11×10^3	5.77×10^4	1.000×10^{-4}	STDEV	9.79×10^0	4.27×10^2	3.512×10^{-4}

a; STDEV: standard deviation

and new analytical method for chlorquinaldol and promestriene marketed in one tablet was developed.

Since the analytical method listed in the Pharmacopeia is applied to medicines that are currently manufactured, they are required to be simple cost-effective and robust. Accordingly, this study took these details into consideration during development of the analytical method. For the analytical instrument, an HPLC-UV was chosen with an ODS, 150 mm length column as it is used more frequently than other length columns and the method can be transferred to longer length columns without the loss of separation. For the mobile phase, methanol and water was chosen as they are cost-effective and widely used. Moreover, separation was done using an isocratic condition for simplicity. In general, if the separation fails, acetonitrile or buffer solution may be replaced instead of methanol and water or gradient condition can be applied as a second choice.

UV absorption wavelength of 220 nm was chosen as both two compounds show marked absorption at this wavelength. With this mobile phase, the two compounds were separated within a short retention time, increasing efficiency. The column did not require a high temperature but to maintain constant temperature, it was set at 30 °C. Samples and standards were both dissolved in methanol in consideration of solubility.

In order to validate the analytical method, the study referred to the validation protocol by KFSA.²⁰ The categories include linearity, precision, accuracy (recovery) and system suitability. And for the system suitability, the repeatability and resolution were considered. Then, the analytical method was validated by assaying marketed products and by conducting an

inter-laboratory validation for reproducibility of the developed analytical method in another laboratory, thus the robustness of the testing method was confirmed.

3.1. Linearity

Three calibration curves were made by analyzing 80, 90, 100, 110 and 120% of the standard solution containing chlorquinaldol and promestriene for linearity (Table 1). The test method had $r^2 \geq 0.999$ for both chlorquinaldol and promestriene.²⁰

3.2. Precision

The precision was aimed at $RSD\% \leq 1$ which is the relative standard deviation between result values by analyzing samples repetitively for the 80, 100 and 120% samples 3 times a day and for 3 consecutive days (Table 2). Based on the data, the RSD ranged between 0.21~0.96% which is less than 1% set by the guidelines 「Guideline on Validation of Analyzing Methods such as Medical Supplies etc.」²⁰

3.3. Accuracy (recovery)

After dissolving chlorquinaldol and promestriene to 80% of the marketed drug, 100, 110 and 120% solutions were made by adding standard (Table 3).

Table 2. Results of precision Intra/inter-day validation of the HPLC-UV method using standard solutions

Validation	Precision (RSD, relative standard deviation: %)					
	Chlorquinaldol			Promestriene		
Contents (%)	80	100	120	80	100	120
Intra-day (n=3)	0.3	0.2	0.5	0.5	0.5	0.8
Inter-day (n=9)	1.0	1.0	0.7	0.8	0.8	0.6

Table 3. Recovery tests for commercial drug (n=3) using HPLC-UV method

Contents (%)	Chlorquinaldol		Promestriene	
	Recovery (%)	RSD ^a (%)	Recovery (%)	RSD ^a (%)
100	98.9	0.7	99.9	0.8
110	99.2	0.7	100.4	0.4
120	99.8	0.7	99.6	0.5

a; RSD: relative standard deviation

Table 4. Results of system validation (n=6)

Contents (%)	Repeatability	
	(RSD, relative standard deviation: %)	
	Chlorquinaldol	Promestriene
100	0.7	0.8

Each was repetitively analyzed 3 times and the recovery rate was calculated by using the calibration curve obtained in the result of linearity test. Accuracy was determined to be 98.9~100.4%.

3.4. System suitability (repeatability & resolution)

System suitability was verified repeatability and resolution. Repeatability was verified by the relative standard deviation (RSD) of the peak area obtained from the 100% solution (chlorquinaldol 200 ppm, promestriene 10 ppm) analyzed repeatedly 6 times (Table 4). Both chlorquinaldol and promestriene met the criteria of less than 1% from 「Guideline on Validation of Analyzing Methods such as Medical Supplies etc.」. With respect to resolution, resolution of both peaks was calculated according to the following equation:

$$Rs = 1.18 \times (t_{Ra} - t_{Rb}) / (W_{1/2a} + W_{1/2b})$$

Rs : Resolution

t_{Ra} , t_{Rb} : Retention time of two materials

$W_{1/2a}$, $W_{1/2b}$: Width of peak at the half position of each peak height

In the HPLC chromatogram (Fig. 2), the retention time of chlorquinaldol was 2.6 min and the retention time of promestriene was 8.7 min. The width at half position of each peak height is around 0.2 min and

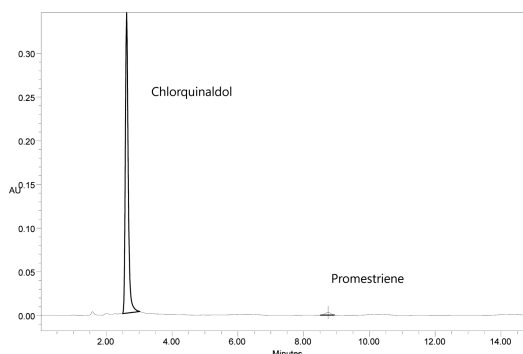


Fig. 2. HPLC-UV chromatogram of chlorquinaldol and promestriene standards.

around 0.5 min, respectively. Accordingly, the resolution of peaks based on the equation is 10.3 and the average of 6 repetitions was around 10.2 with a standard deviation of 0.06. Based on this, the 95% confidence interval of peak resolution for chlorquinaldol/promestriene tablet is 10.16~10.28, which is suitable based on the criterion of 1.5 from 「Guideline on Validation of Analyzing Methods such as Medical Supplies etc.」.

3.5. Limit of Detection (LOD) and Limit of Quantification (LOQ)

LOD and LOQ were computed to verify the lowest quantity of compounds that could be discriminated and the lowest quantity of compounds that can be acquired quantitatively, respectively. These numerical values were calculated using following equation 1 and 2. σ represents the standard deviation of intercept from the linearity calibration curve and S represents the slope of calibration curve. As a result, the LOD and LOQ values of Chlorquinaldol were 7.2 ppm and 21.6 ppm. And, the LOD and LOQ values of Promestriene were 1.4 ppm and 4.2 ppm, respectively.

$$LOD = 3.33 \times \frac{\sigma}{S} \quad (1)$$

$$LOQ = 10 \times \frac{\sigma}{S} \quad (2)$$

3.6. Inter-laboratory validation

The identical analytical method was reproduced in three institutions including the one which developed

Table 5. Inter-laboratory validation results

	Chlorquinaldol		Promestriene	
	Content (%)	RSD ^a (%)	Content (%)	RSD ^a (%)
Lab A	99.3	0.7	100.0	0.6
Lab B	100.8	0.5	98.2	1.0
Lab C	102.5	0.8	104.1	0.7

a; RSD: relative standard deviation

the method. Therefore, consistency between laboratory environments, equipment and personnel was assessed. For chlorquinaldol and promestriene, calibration curves consisting of 80, 90, 100, 110 and 120% of the marketed concentrations were analyzed as well as the commercial product (100% strength). The contents of the commercial products were quantified and this was repeated three times to calculate relative standard deviation (Table 5). Content 99.3~104.1% and RSD% ≤ 1.0 were calculated, confirming the robustness of the testing method.

4. Conclusion

In this study, a validated analytical method which can identify and quantify chlorquinaldol and promestriene from a marketed drug in one assay was developed. Thus, in the future, this method may be applied for quality control guideline in other laboratories in a cost-effective and efficient way.

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