11th Joint Conference on Chemistry in Conjunction with the 4th Regional Biomaterials Scientific Meeting

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INTERNATIONAL CONFERENCE

11th Joint Conference on Chemistry

in conjunction with

The 4th Regional Biomaterial Scientific Meeting

"Material Chemistry Development for Future Medicine, Industry, Environmental and Biomaterial Application"

September, 15 - 16, 2016
Purwokerto - Indonesia

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Welcome Message From Chairman of Conference

On behalf of the Committee, I am very pleased that the 11th Joint Chemistry Conference in Conjunction with The 4th Regional Biomaterials Scientific Meeting has attracted many scientist from Indonesia, Malaysia, Thailand, Bangladesh, Egypt, Japan as well as other countries.

This international conference is attended by more than 150 participants covering wide variety subject grouped as theoretical chemistry and educational, material synthesis and modification, bioscience and analysis, and also Industrial and environmental chemistry. The given oral and poster presentation would showing outputs for future need as indicated in the conference theme of “Material Chemistry Development for Future Medicine, Industry, Environmental and Biomaterial Application”

The success of the Conference would not have been attained without strong supports from contributing scientists and our partner institutes including Diponegoro University, Semarang State University, Sebelas Maret University, Satya Wacana Christian University and Indonesian Biomaterial Society. I would like to thank all of them for helping to make a very successful conference.

Chairman,

Amin Fatoni, Ph.D

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Development of high performance liquid chromatography method for miconazole analysis in powder sample

Development of high performance liquid chromatography method for miconazole analysis in powder sample

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Abstract. A simple high performance liquid chromatography (HPLC) method has been developed in this study for the analysis of miconazole, an antifungal drug, in powder sample. The optimized HPLC system using C_8 column was achieved using mobile phase composition containing methanol:water (85:15, v/v), a flow rate of 0.8 mL/min, and UV detection at 220 nm. The calibration graph was linear in the range from 10 to 50 mg/L with r^2 of 0.9983. The limit of detection (LOD) and limit of quantitation (LOQ) obtained were 2.24 mg/L and 7.47 mg/L, respectively. The present HPLC method is applicable for the determination of miconazole in the powder sample with a recovery of 101.28 % (*RSD* = 0.96%, *n* = 3). The developed HPLC method provides short analysis time, high reproducibility and high sensitivity.

1. Introduction

Many types of drugs are produced synthetically and commercially available in dosage forms. One example of important drugs used as a medication is antifungal drugs. An antifungal drug is used to treat deep infections caused by a fungus. This drug can be divided into several classes, including triazoles and imidazoles. Miconazole is an imidazole antifungal agent (figure 1). It is commonly used to the skin or to mucous membrane to cure fungal infections, due to its high therapeutic properties. It has been extensively applied in the management of dermal, oral and vaginal mycosis. It is used in a variety of pharmaceutical formulations such as injections, tablets, oral gels, creams, ointments, topical powders and vaginal suppositories. The most usual application forms include creams, ointments or gels at 2.0 % concentration level, alone or associated with anti-inflammatory steroids, or other antimicrobials such as gentamicin for the treatment of dermatitis. Diaper dermatitis, a common dermatologic disorder in infancy, frequently associated with Candida albicans infections, is currently treated with ointments containing 0.25 % miconazole nitrate [1-2].

Chromatographic methods have achieved a great reputation in separation science; mainly using high performance liquid chromatography (HPLC) method [3-10]. The chromatographic process can be described as a separation technique that includes mass-transfer between stationary and mobile phase. HPLC uses a liquid mobile phase to separate the components in a mixture. The stationary phase can be in a liquid phase or a solid phase. These components are forced to flow through a chromatographic column under high pressure after being dissolved in a solvent. The mixture is then separated into its

components. In our previous study, the HPLC method with ultraviolet (UV) detector has been successfully developed for the determination of miconazole nitrate in a cream sample using C_{18} column and ACN:MeOH (90:10 v/v) as mobile phase [11]. The inherent ability of HPLC for the quantification of drugs possessing good efficiency, reproducibility, selectivity and sensitivity combined with the ease of method development and rapid analysis make it a very good alternative for drugs analysis.

It is thus of interest in this study to develop the HPLC method for miconazole analysis in the different pharmaceutical sample. The developed HPLC method using C_8 column and MeOH:water as the mobile phase is effective, fast and reproducible for the determination of miconazole in the powder sample.

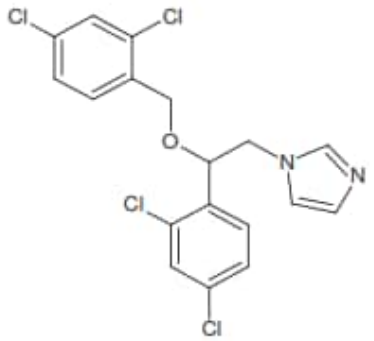

Figure 1. Chemical structure of miconazole

2. Method

2.1 Materials

Miconazole was obtained from Sigma (St. Louis, MO, USA). Methanol used is HPLC grade. Deionized water was taken from a Millipore Simplicity. All solvents were degassed prior to usage. Powder sample was obtained from local drug market. A stock solution of miconazole (1000 mg/L) was prepared by dissolving miconazole standard in methanol. A series of standard working solution with different concentrations ranging from 10 to 50 mg/L was prepared by further dilution of the stock solution. The stock and all standard working solutions were labeled and sealed with aluminum foil to avoid evaporation, and were stored in the refrigerator prior to use. The powder sample containing 2% of miconazole were weighed. It was sonicated in 10 mL methanol for 10 minutes. The extract was allowed to cool and then was filtered. The volume of the sample solution was adjusted to 25 mL with methanol. Finally, the sample was injected to the HPLC-UV method.

2.2 HPLC Instrument

The HPLC system used was Hitachi L-2000 series (Japan), equipped with a Model L-2130 pump, an on-line solvent vacuum degasser, an autosampler with 5 μL injection loop and a UV-Vis detector L-2420. The separation was carried out in a Phenomenex C8 column Luna 10μ (150 x 4,6 mm). The mobile phase consisted of methanol:water (85:15, v/v). The system was operated isocratically at flow rate 0.8 min/mL and UV wavelength 220 nm.

3. Results

3.1 Optimization of Mobile Phase

Effect of different composition of mobile phase (methanol:water) was first explored in this study to obtain the optimum HPLC condition. The mobile phase composition was varied in three different

percentages of methanol-water (v/v): (85:15), (90:10), and (95:15). The typical result of HPLC chromatogram for miconazole with different composition of the mobile phase is shown in figure 1. It can be seen that the retention time of miconazole was gradually increased when the percentage of water was increased. Addition of water reduces the elution strength of the mobile phase; therefore the analyte was eluted slower. Methanol-water (85:15, v/v) has been chosen as the optimized mobile phase composition because it gave the best baseline of miconazole peak (base to base) with retention time less than 7 min.

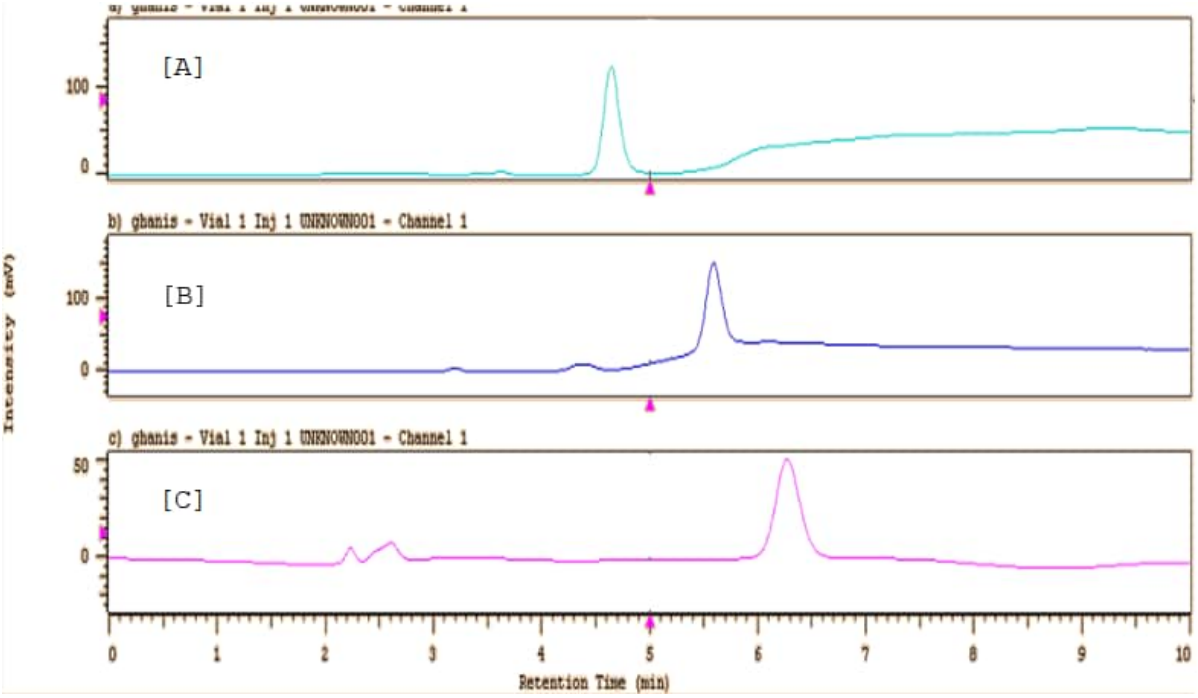

Figure 2. Chromatogram of miconazole (50 mg/L in methanol) by HPLC with different mobile phase composition. (A) methanol-water (95:5, v/v); (B) methanol-water (90:10, v/v); (C) methanol-water (85:15, v/v). HPLC conditions; flow rate: 0.8 mL/min, C_8 column (150 x 4,6 mm), UV detector (220 nm), injection volume: 5 µL

3.2 Optimization of Flow Rate

The effect of different flow rate of mobile phase was then investigated in this study. Flow rate was varied in three different flow rates: 0.7, 0.8 and 0.9 mL/min. The typical result of HPLC chromatogram for miconazole with a different flow rate of the mobile phase is shown in figure 2. It can be seen that retention time of miconazole was gradually increased when the flow rate of mobile phase was decreased. The 0.8 mL/min has been chosen as the optimized flow rate because it gave the best baseline of miconazole peak (base to base) with retention time less than 7 min.

3.3 Optimization of UV Detector Wavelength

The effect of different UV detector wavelength was also investigated in this study. UV detection was varied in three different wavelengths: 210, 220 and 230 nm. The typical result of HPLC chromatogram for miconazole with a different wavelength is shown in Fig 3. It can be seen that retention time of miconazole was not significantly different. UV detector wavelength 220 nm has been chosen as the optimized wavelength because it gave the best baseline of miconazole peak (base to base) with retention time less than 7 min.

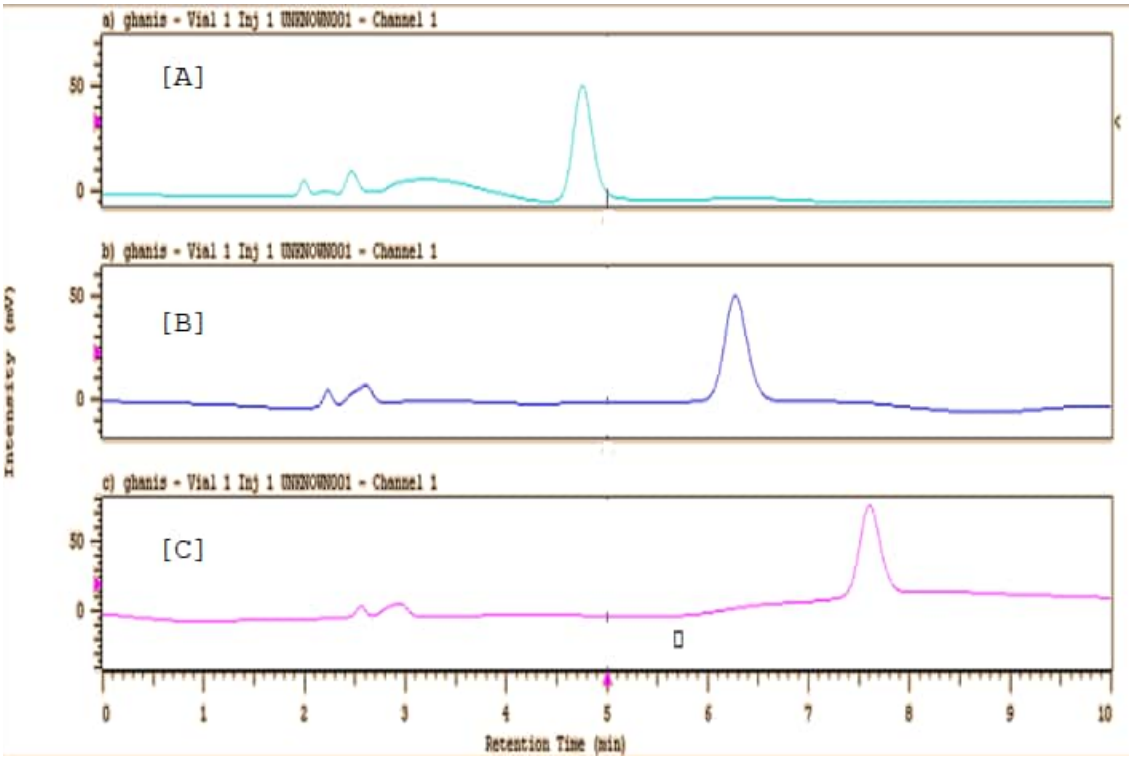

Figure 3. Chromatogram of miconazole (50 mg/L in methanol) by HPLC with different flow rate. (A) 0.9 mL/min; (B) 0.8 mL/min; (C) 0.7 mL/min. Mobile phase composition, methanol-water (85:15, v/v); other conditions: as shown in figure 2.

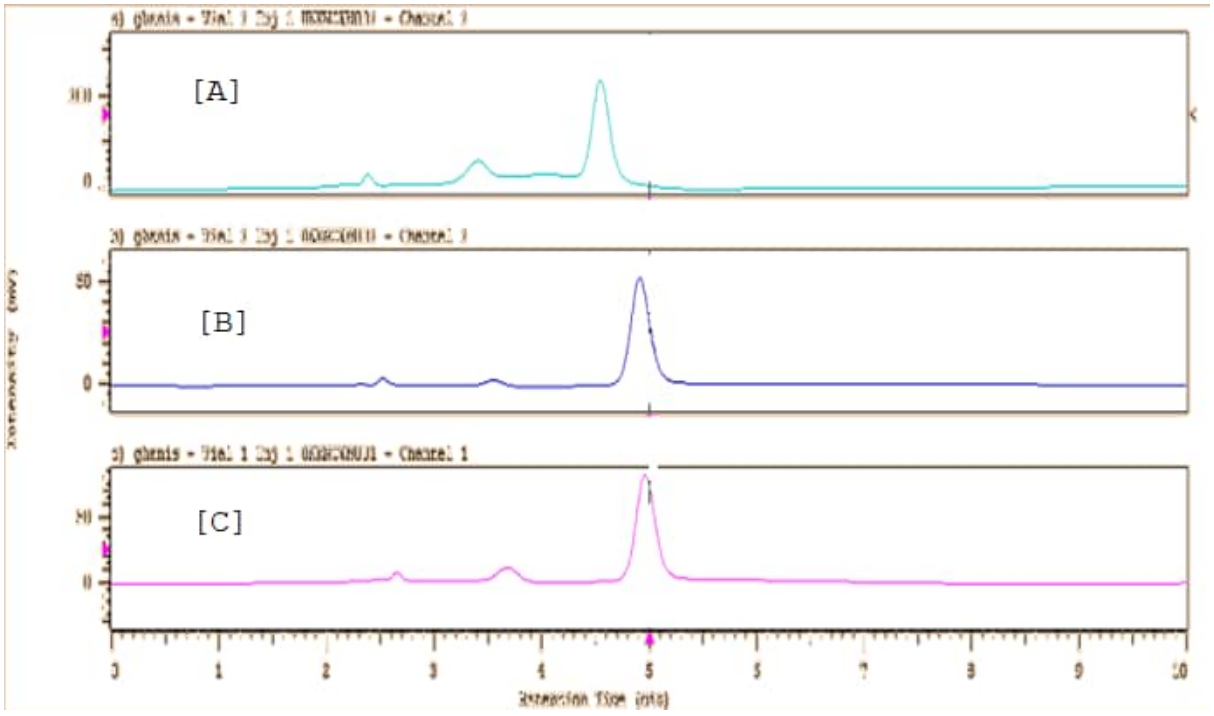

Figure 4. Chromatogram of miconazole (50 mg/L in methanol) by HPLC with different wavelengths. (A) 210 nm; (B) 220 nm; (C) 230 nm. Mobile phase composition, methanol-water (85:15, v/v); flow rate 0.8 mL/min. Other conditions: as shown in figure 2.

3.4 Analytical Performance of the HPLC Method

The performance of the developed HPLC method was examined in term of linearity, limit of detection (LOD), limit of quantitation (LOQ), recovery and repeatability (relative standard deviation/RSD). The calibration curve was linear for miconazole from 10 to 50 mg/L with r^2 = 0.9983. The LOD and LOQ of miconazole were 2.24 mg/L and 7.47 mg/L, respectively. The low LOD and LOQ showed that the developed HPLC method is sensitive and sufficient to determine miconazole in pharmaceutical samples for routine analysis. Average recoveries of miconazole in powder sample was calculated by comparing the peak height obtained from injections of miconazole standard with those obtained by injections of the samples with known concentration of miconazole drug. The percentage recovery obtained for analysis of miconazole in powder sample was 101.28% with RSD of 0.96% (n = 3).

4. Conclusions

In the present work, the high performance liquid chromatography (HPLC) method using C_8 column was successfully developed for the determination of miconazole in the pharmaceutical sample (powder). The optimum HPLC condition was achieved using mobile phase composition containing methanol:water (85:15, v/v), the flow rate of 0.80 mL/min, and UV detection at 220 nm. The HLPC condition gave retention time of miconazole within 7 minutes. The calibration graph was linear in the range from 10 to 50 mg/L with r^2 of 0.9983. The limit of detection (LOD) and limit of quantitation (LOQ) obtained were 2.24 mg/L and 7.47 mg/L, respectively. The optimized HPLC method was successfully applied to the analysis of miconazole in the pharmaceutical sample (powder). The percentage recovery obtained for analysis of miconazole in powder sample was 101.28 % with RSD of 0.96 % (n = 3).

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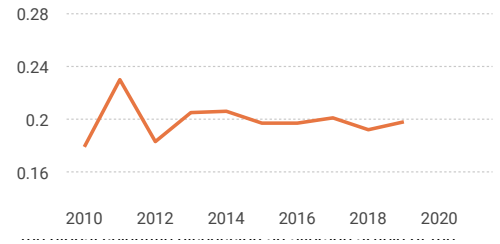

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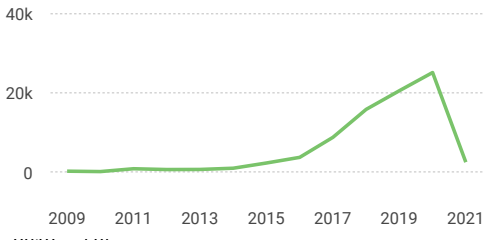

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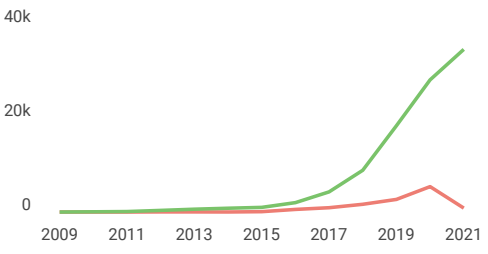

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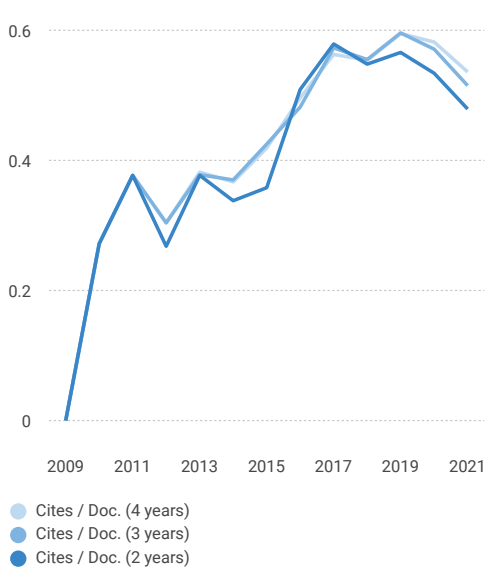

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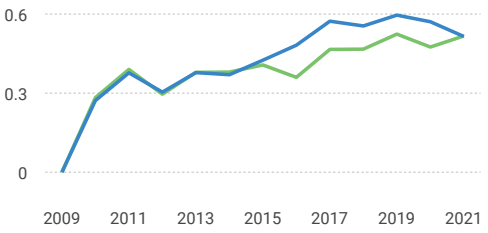

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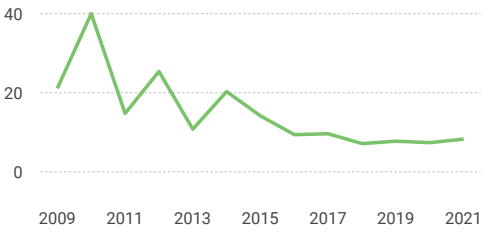

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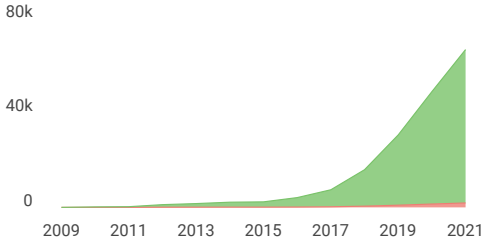

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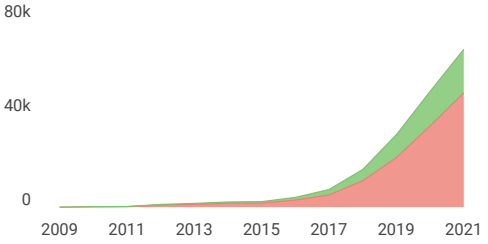

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