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Dear participant,

ACCEPTANCE FOR THE MALAYSIA SEPARATION SCIENCE CONFERENCE 2017 (MYSSC 2017) FROM 23 - 25 OCTOBER 2017

Thank you for accepting our invitation as Invited Speaker in the upcoming MySSC 2017. We are pleased to inform you that your proposed paper entitled "Application of chiral and non-chiral HPLC method for selected antifugal drug analysis in the pharmaceutical formulation" has been accepted and your registration code is IS-007.

We appreciate all support given for the success of MySSC 2017. Kindly proceed to fee payment which can be made through our website (http://www.utm.my/myssc/payment/) . Please be noted that the:

- Early bird registration & payment deadline is 15 September 2017
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Thank you and we look forward to seeing you in Johor Bahru.

Yours sincerely,
Wan Aini Wan Ibrahim, PhD
Conference chair

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APPLICATION OF CHIRAL AND NON-CHIRAL HPLC METHOD FOR SELECTED ANTIFUNGAL DRUG ANALYSIS IN THE PHARMACEUTICAL FORMULATION

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Abstract

High performance liquid chromatography (HPLC) method using chiral and non-chiral columns has been successfully applied in this study for selected antifungal drug (ketoconazole) analysis in the pharmaceutical samples. Three HPLC parameters were optimized including the mobile phase composition, flow rate and wavelength. The optimum HPLC conditions were obtained at mobile phase composition containing acetonitrile:water (80:20, v/v), flow rate of 0.60 mL/min with UV detection at a wavelength of 235 nm. The optimized condition gave the retention time for ketoconazole within 4.3 min. An excellent linearity was obtained with r^2 of 0.9996 ranging from 1 to 10 mg/L of ketoconazole. The limit of detection (LOD) and limit of quantitation (LOQ) calculated were 0.23 mg/L and 0.77 mg/L, respectively. The average recovery of ketoconazole found in tablet sample was 80.33% (RSD = 0.97%). In addition, chiral separation of two ketoconazole enantiomers was obtained by the HPLC method using CYCLOBOND I 2000 HP-RSP (25 cm x 4.6 mm x 5 μm) column; mobile phase composition of acetonitrile:water (0.2% HCOOH) 20:80; 1.0 mL/min flow rate; and UV detection at 220 nm with resolution (Rs) = 1.66. The calibration graph was linier in the range 25 – 100 mg/L with correlation value (r) of 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) obtained were 2.98 and 10.06 mg/L, respectively. Determination of ketoconazole in cream sample was studied with recovery of 102.10% (RSD= 0.31%). This HPLC method was simple, rapid analysis and high resolution.

Key words: Antifungal drug, Ketoconazole, HPLC, Pharmaceutical sample, Urine sample.

Introduction

Drugs are crucial in human lives as they are used as medication to treat diseases. Nowadays, many types of drugs are produced synthetically and commercially available in dosage forms [1]. One example of important drugs used as medication is antifungal drugs. Antifungal drug is used to treat deep infections caused by a fungus. This drug can be divided into several classes, but the common ones are triazoles and imidazoles. Ketoconazole (**Figure 1**) is a class of imidazole which has antifungal activity, where the compound interacts with the cytochrome P450 enzyme, 14 α-demethylase by converting lanosterol to ergosterol which is an important component of fungal cell membranes which inhibits the synthesis process.

In the pharmaceutical analysis of ketoconazole, high performance liquid chromatography (HPLC) technique has been widely used [2-5] as well as in the analysis of biological fluids. The application of HPLC in analysis is preferable because it provides shorter analysis time, high resolution, selectivity and sensitivity. Chiral separation has been widely studied in the synthesis of organic compounds, both in the chemical and pharmaceutical fields (6-10). This separation is done in order to get one of the enantiomers that has active properties as a drug, because a stereoisomer besides having an active side also has an inactive or even toxic side when consumed.

Separation of chiral compounds in ketoconazole samples is still rare. This study explains the validation of the separation of chiral compounds in ketoconazole cream and tablet samples. One method that can be used in chiral separation is HPLC. In this study, HPLC method with ultraviolet (UV) detector has been developed for quantitative determination of ketoconazole in tablet sample. In addition, the HPLC method using cyclodextrin as chiral column was also successfully applied for chiral separation and determination of ketoconazole in the cream sample.

Material and Methods

Chemicals and Reagents

Ketoconazole was obtained from Sigma (St. Louis, MO, USA). Acetonitrile and methanol used are HPLC grade. Deionized water was taken from a Millipore Simplicity (Simpak®2). All solvents were degassed prior to usage.

Instrumentation

The HPLC system used was Agilent 1100 Series (Germany), equipped with a Model Agilent 1100 pump, an on-line solvent vacuum degasser, an auto sampler with 20 μL injection loop and an Agilent 1100 Series UV detector. The separation was carried out in a PHENOMENEX C_{18} column (150 mm x 4.6 mm x 5 μm particles). The mobile phase consisted of acetonitrile:water (80:20, v/v). The system was operated isocratically at flow rate 0.60 min/mL and at UV wavelength 235 nm. An Agilent 1100 Series UV Spectrophotometer (Germany) was used to record the maximum absorption of the analyte.

The HPLC system using CYCLOBOND I 2000 HP-RSP (25 cm x 4.6 mm x 5 μm) column; mobile phase composition of acetonitrile:water (0.2% HCOOH) 20:80; 1.0 mL/min flow rate; and UV detection at 220 nm was used for chiral separation of ketoconazole.

Preparation of Standards

A stock solution of ketoconazole (2000 ppm) was prepared by dissolving 0.02 g of ketoconazole in methanol. A series of standard working solution with four different concentrations ranging from 1.0 to 10.0 ppm was prepared by further dilution of the stock solution. The stock and all standard working solutions were labeled and sealed with aluminium foil to avoid evaporation, and were stored in the refrigerator prior to use.

Preparation of Samples

Triplicate of ketoconazole tablet sample containing 200 mg of ketoconazole were weighed and powdered. The powdered sample was transferred into a 100 mL volumetric flask and was shaken with 70 mL of methanol. The sample was ultrasonicated for 30 minutes and the extract was allowed to cool. The volume of the sample was adjusted to 100 mL with methanol. Then, the solution was filtered.

About 0.25 mL of the aliquot was added to a 10 mL volumetric flask and the volume was adjusted with methanol. Further dilution of the standard solution was done with methanol to obtain a 5 ppm ketoconazole tablet sample solution. Finally, the sample was injected to the HPLC-UV method.

Cream sample preparation was carried out using the SPE method taken from previous studies with several modifications. Cream sample of ketoconazole was prepared by dissolving ketoconazole cream (2% ketoconazole) in 5 mL dichloromethane. Furthermore, sonicated for 5 minutes, then dichloromethane is added to 25 mL. The solution was sonicated for 15 minutes, then filtered. SPE diol column was activated by preconditioning with 6 mL dichloromethane. A sample solution of 1 mL was put into the SPE cartridge under vacuum. The sample solution was rinsed using 3 mL n-hexane:dichloromethane (4:1 v/v) 2 times and the column was dried for 5 minutes. Then, the analyte was eluted with 1 mL of methanol 3 times and methanol was added to 10 mL. Furthermore, the solution was used for analysis with HPLC.

Results and Discussion

Effect of Mobile Phase Composition on Retention Time

The retention time of ketoconazole will be longer when the percentage of water was higher than organic solvent (data not shown). Addition of water reduces the elution strength of the mobile phase, therefore the analyte was eluted slower. The acetonitrile-water offers shorter retention time than methanol-water. This is because the combination of acetonitrile and water generally has greater elution strength compared to the combination of methanol and water of the same ratio.

Effect of Mobile Phase Composition on Peak Area

The peak area of ketoconazole was evaluated using different composition of water in the mobile phase. For both acetonitrile-water and methanol-water, it shows that as the composition of water was increases, the peak area decreases. This means that more amount of analyte was detected when higher composition of organic solvent was used. It also can be seen that mobile phase

acetonitrile- water gives higher peak area compared to methanol-water (data not shown).

Effect of Mobile Phase Composition on Peak Height

The peak height of ketoconazole at different composition of water in the mobile phase was also investigated. The peak height of acetonitrile-water and methanol-water decreases as the percentage of water in the mobile increases (data not shown). The same trend was observed at mobile phase of 100 % acetonitrile (no water in the mobile phase composition) where the peak area was very low due to the low solubility of ketoconazole in acetonitrile. Acetonitrile-water offers greater peak height compared to methanol-water. Therefore, mobile phase acetonitrile-water (80:20, v/v) has been chosen as the optimized composition compared to methanol-water because it gives shorter retention time, and greater peak area and peak height.

Optimization of Flow Rate

In this study, the flow rate has been optimized using 3 different flow rates; 0.60, 0.80 and 1.0 mL/min. From the analysis, the optimized flow rate for acetonitrile-water was 0.60 mL/min. It was chosen because it gives higher peak area and peak height compared to flow rates of 0.80 and 1.0 mL/min with relatively short retention time.

Optimization of UV Detector Wavelength

At wavelength 254 nm and 239 nm, analyte peak with lower peak area and peak height were obtained. As the wavelength was reduced to 235 nm, it gives higher peak area and peak height. However, further reduction of the wavelength to 230 nm resulted in detection of other substances in the compound (interference). Therefore, 235 nm was chosen as the optimized wavelength because it offers shorter retention time, and greater peak area and peak height compared to other wavelength tested.

Linearity Data

The calibration curve was linear from 1 to 10 ppm and shows a good coefficient of determination, r^2 of 0.9996. The limit of detection and limit of quantitation of ketoconazole was 0.23 ppm and 0.77 ppm respectively.

Recovery

The average recoveries of ketoconazole in tablet and urine samples was calculated by comparing the peak height obtained from the injections of ketoconazole standard with those obtained from the injections of the samples with known concentration of ketoconazole drug. For the tablet sample, the average recovery obtained was 80.33 % with RSD of 0.974 % (n=3). Figure 2 shows the HPLC chromatogram of ketoconazole in the sample.

Enantioseparation of ketoconazole

In this study, the HPLC system using CYCLOBOND I 2000 HP-RSP (25 cm x 4.6 mm x 5 μm) column; mobile phase composition of acetonitrile:water (0.2% HCOOH) 20:80; 1.0 mL/min flow rate; and UV detection at 220 nm could separate ketoconazole enantiomers with resolution (*Rs*) greater than 1.50. The calibration graph was linier in the range 25 – 100 mg/L with correlation value (r) of 0.9997. The limit of detection (LOD) and limit of quantification (LOQ) obtained were 2.98 and 10.06 mg/L, respectively. Determination of ketoconazole in cream sample was studied with recovery of 102.10% (RSD= 0.31%). The value of selectivity (Figure 3) was studied from the injection of ketoconazole and econazole ($\propto$ = 1.29).

Conclusion

A high-performance liquid chromatography (HPLC) method for analysis of ketoconazole in pharmaceutical sample (tablet) has been developed. The optimum HPLC condition was achieved using mobile phase composition containing acetonitrile:water (80:20, v/v), flow rate of 0.60 mL/min, UV detection at 235 nm and 20 μL sample injection. The HLPC condition gave retention time of ketoconazole at 4.2 minutes. The percentage recovery obtained for analysis of ketoconazole in tablet sample was 80.33 % with RSD of 0.974 % (n=3). In addition, the HPLC method using cyclodextrin as chiral column was also successfully applied for chiral separation and determination of ketoconazole in the cream sample.

Acknowledgement

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Figures

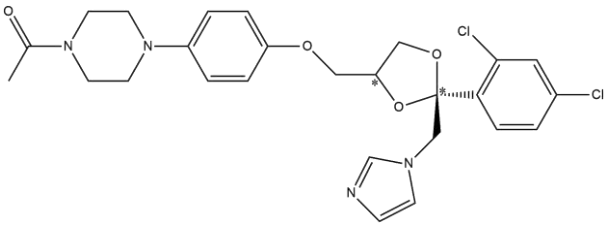

Figure 1. Chemical structure of ketoconazole

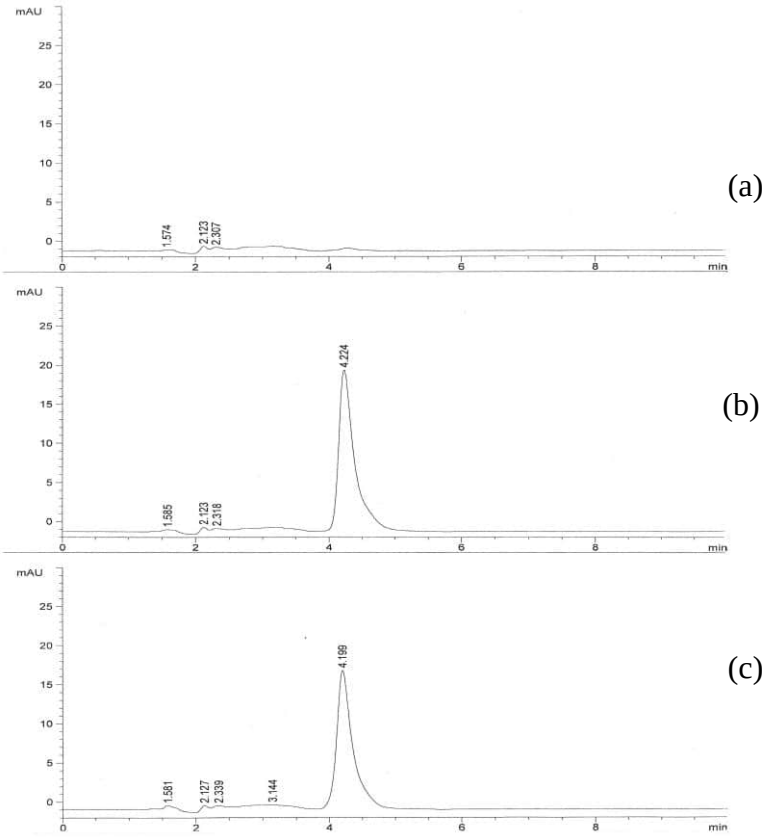

Figure 2. HPLC chromatogram of (a) methanol, (b) ketoconazole standard (5 mg/L), and (c) tablet sample. HPLC conditions; acetonitrile-water (80:20, v/v), C_{18} column (150 mm × 4.6 mm × 5 μm), flow rate of 0.60 mL/min, UV detector of 235 nm, injection volume of 20 μL.

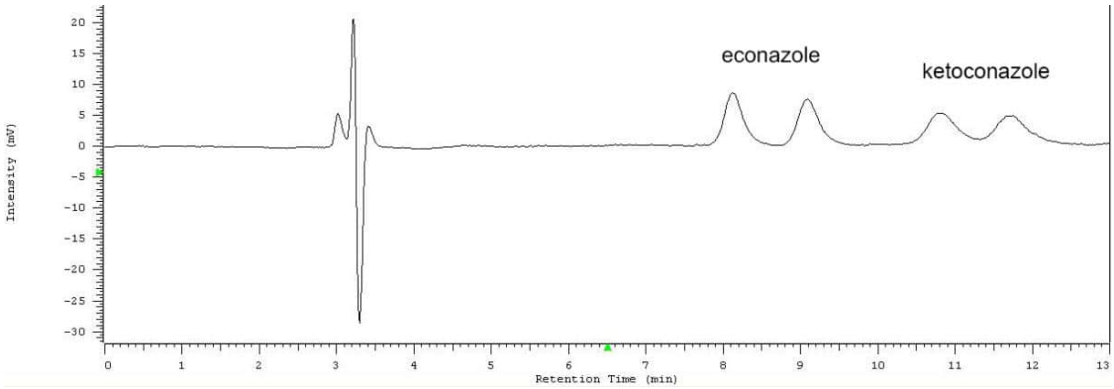

Figure 3. HPLC chromatogram of enantioseparation of ketoconazole and econazole (50 mg/L) using CYCLOBOND column (I 2000 HP-RSP, 5 µm) size 25 cm x 4,6 mm; mobile phase of acetonitrile:0.2% formic acid (20:80 v/v); UV detector of 220 nm; flow rate of 1,0 mL/min; and volume injection of 1 µL.