28st June 2018

Dear **Dadan Hermawan**

Jenderal Soedirman University, Indonesia

ACCEPTANCE LETTER

Thank you for submitting your abstract for presentation at "13th Joint Conference on Chemistry" to be held 7th -8th September 2018 in Semarang, Indonesia. We have reviewed your paper abstract and found that it meets preliminary acceptance requirements set forth by the committee

Your abstract entitled:

1. **Chiral separation of miconazole by high performance liquid chromatography and micellar electrokinetic chromatography** is accepted for **Oral Presentation**

Congratulations and you may begin making your conference plans!

Please save this message for reference.

- ✓ Confirmation of your presentation on the final schedule is contingent upon full payment of fees by 7th July 2018 for early bird and 7th August for late payment.
- ✓ After that time, your presentation will be dropped from the program if you are not yet registered with fees paid in full. If you have extenuating circumstances which would require later payment, please contact us jcc@live.undip.ac.id to make arrangements prior to 7th August 2018.
- ✓ The registrar will provide confirmation when your registration and fees have been received and processed.
- ✓ Please note that 13th Joint Conference on Chemistry committee does not have funds available to pay registration and/or travel expenses for program participants.
- ✓ You must attend and present the paper at the conference in order to be included in the program and proceedings.
- ✓ The preliminary program, including registration materials, will be posted online at conference website (https://jcc.undip.ac.id/)
- ✓ Substitutions for presenting authors may be made until 7th August 2018. The substitute must be registered for the conference by contacting conference committee. The substitute will be listed as the presenting author in conference materials.
- ✓ No changes to the title or text of your submission will be accepted after 7th August 2018.
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- ✓ Please note that individual requests for specific presentation dates and/or times cannot be addressed due to the very large number of presentations and other factors that the program committee must consider when scheduling the overall program.
- ✓ You are encouraged to prepare a fullpaper to be published, however the submission of a paper is optional.

- ✓ A review coordinator will arrange for two independent and anonymous reviews. Papers deemed acceptable by this process will be included in the conference proceedings or conference partner journal.
- ✓ Papers must be no longer than 8,000 words and must be written in English prepared in Word and submitted by 14th August 2018.
- ✓ Reviews will be completed and comments sent back to you by 7th October 2018. Revisions, if applicable, will be due 21st October 2018.
- ✓ Please note that you must be registered, with fees paid, and you must make your presentation at the conference in order to have your paper published in the conference proceedings or conference partner journal.

Feel free to contact us (jcc@live.undip.ac.id) if you have any questions about your abstract and full paper submission. We try to respond to you quickly

Thank you for your interest, and we look forward to working with you on a successful conference

Yours sincerely,

Adi Darmawan, Ph.D
The Chair of 13th Joint Conference on Chemistry

13th Joint Conference on Chemistry

Certificate of Presentation

This is to certify that

Dadan Hermawan

has presented a paper entitled

Chiral Separation of Miconazole by High Performance Liquid Chromatography and Micellar Electrokinetic Chromatography

at the **13th Joint Conference on Chemistry** held on 7-8 September 2018 in Semarang that organised by Chemistry Department, Diponegoro University

Semarang, 8 September 2018

Dean of Faculty of Sciences and Mathematics
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Chiral Separation of Miconazole by High Performance Liquid Chromatography and Micellar Electrokinetic Chromatography

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Chiral separation is one of the important fields of modern analytical chemistry, since it is well known that the enantiomers (optical isomers) differ in their pharmacological and toxicological activities. Chiral separation of miconazole, an antifungal drug with one chiral center, has been developed in this study using the high-performance liquid chromatography (HPLC) and micellar electrokinetic chromatography (MEKC) methods, respectively. In the preliminary study, enantioresolution of miconazole (Rs = 1.0) was achieved by the CD-MEKC system containing 40 mM HP-γ-CD (as chiral selector), 40 mM SDS (as surfactant), 20 mM phosphate buffer (pH = 8) and 5% acetonitrile. The CD-MEKC method was simple, less solvent used, and relatively short analysis time (within 10 min). In addition, the best enantioresolution of miconazole (Rs = 2.2) was achieved in this study by the HPLC system using Cyclobond I 2000 HP-RSP as chiral column (25 cm × 4.6 mm × 5 μm), acetonitril:water (0.1% HCOOH) 20:80 as mobile phase, flow rate of 1.0 mL/min, and detection wavelength at 230 nm. The present HPLC method was simple, high resolution and rapid analysis.

Keywords: Chiral separation; Miconazole; High performance liquid chromatography; Micellar electrokinetic chromatography.

Introduction

Chiral separation are concerned with separating the pairs of enantiomers or optical isomers, which can exist as nonsuperimposable mirror images. Enantiomers have identical chemical properties except in their reactivity toward optically active reagents. They are identical in all physical properties except for the direction in which they rotate the plane of polarized right. They rotate the plane of polarized light in opposite directions but with equal magnitude. Many compounds of biological and pharmacological interest are asymmetric and show optical activity. It is well established that the pharmacological activity is mostly restricted to one of the enantiomers (eutomer). In several cases, unwanted side effects or even toxic effects may occur with the inactive enantiomer. The administration of pure, pharmacologically active enantiomers is great importance. The ideal way to get to pure enantiomers would be by enantioselective synthesis. However, this approach is usually expensive and not often practicable. Usually, the racemates are obtained in a synthesis, and the separation of the enantiomers on a preparative scale is necessary. On the other hand, there is also a great demand for methods of enantiomer separation on an analytical scale for controlling synthesis, checking for racemization processes, controlling enantiomeric purity, and for pharmacokinetic studies [1].

Chromatograpic methods have been used for enantiomer separations, where chiral stationary phase or chiral columns are often used to achieve optical recognitions. Several chiral additives to mobile phases are also used instead of chiral stationary phases in high performance liquid chromatography (HPLC) method. In recent years, HPLC has attracted increasing interest for chiral separation, both on analytical and preparative scales. The advantages of HPLC method is versatile, high resolution, high sensitivity and rapid analysis [2-3]. In addition, capillary electrophoresis (CE) has also proven useful for chiral separation on an analytical scale. The advantages of CE method for enantiomer separation are rapid method development, low consumption of analytes, minimal use of expensive chiral reagents and short analysis time [4 – 7]. A fast-growing number of studies are reported on the use of capillary electrophoresis (CE) in chiral separation [8 – 10].

Miconazole, an imidazole fungicide, has a 1,2-diazole ring and consists one chiral center (Fig 1). It is used for the treatment of topical fungal infection in a variety of pharmaceutical formulations. In order to achieve the best chiral separation of miconazole enantiomers, this work focused on the optimization of CD-MEKC method with different organic modifiers. Effects of organic modifier concentration such as acetonitrile and methanol on the enantioresolution of miconazole were investigated in this study. In addition, the HPLC

method with cyclodextrin-based Astec Cyclobond (I 2000 HP-RSP, 25 cm x 4,6 mm, 5 µm) as a chiral selector was also investigated to separate the two miconazole enantiomers.

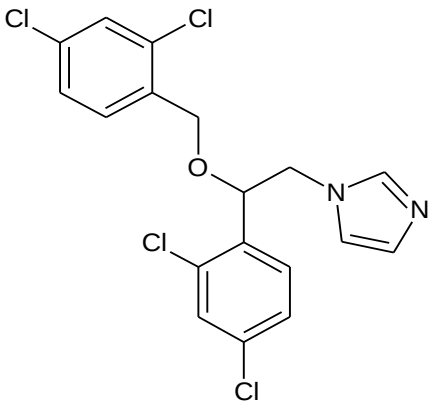

Figure 1. Chemical structure of miconazole

Material and Methods

Chemicals and Reagents

Standard of miconazole nitrate was purchase from Sigma-Aldrich (St. Louis, USA). Sodium dodecyl sulfate (SDS) was purchased from Fischer Chemicals (Loughborough, UK), sodium hydroxide, boric acid and sodium tetraborate anhydrous were purchased from Merck (Darmstadt, Germany). Methanol, butan-1-ol, ethyl acetate and acetonitrile all of analytical HPLC grade were purchased from RCI Labscan (Bangkok, Thailand). Deionized water (18 MΩcm) was purified by Millipores Water Purification System (Molsheim, France). Stock solution (2000 µg/mL) of miconazole nitrate was prepared with methanol as solvent. Working standards solutions were prepared by diluting the stock solutions with methanol. The stock solutions and working solutions were stored in the refrigerator at 5ºC.

Instrumentation of Capillary Electrophoresis

An Agilent Capillary Electrophoresis System (Agilent Technology, Waldbronn, Germany) has been used equipped with diode array detection (DAD) operating at 200 nm. An uncoated fused- silica capillary of 50 µm inner diameter (I.D) with a total length of 64.5 cm (56 cm to detector have been used for the separation processes). The capillary temperature was optimized. In all cases, hydrodynamic injection was used at 50 mbar for 5 s at the capillary inlet to optimize the separation. For every new capillary used, 30 min of conditioning process

with 1.0 M NaOH has been done before it was used. Then, equilibrate with Mili-Q water for 30 min, followed by 0.1 M NaOH for 15 min, and Mili-Q water again for 15 min and finally with the microemulsion background electrolyte (BGE) solution for 10 min. The capillary was pre-conditioned for 2 min with the microemulsion solution and post-conditioned with 0.1 M NaOH and Mili-Q water for 2 min between analysis. When the capillary is not in use, it was flushed in air for 5 min and stored.

Instrumentation of HPLC

The HPLC system used is Hitachi L-2000 series (Japan), equipped with a Model L-2130 pump, an on-line solvent vacuum degasser, an auto sampler with 10 L injection loop and an UV-Vis detector L-2420. The separation will be carried out using selected chiral column. The mobile phase consisted of methanol:water or acetonitrile:water with different percentage. The system is operated isocratically at the selected flow rate and UV wavelength. The detector was set to operate at 230 nm wavelength. Astec CYCLOBOND column (I 2000 HP-RSP, 5 μm) size 25 cm x 4.6 mm) will be evaluated as chiral stationary phase for its suitability to separate enantiomers of selected drug. The resolution (*Rs*) will be calculated from the retention time of peaks according to the equation:

$$\boldsymbol{Rs = (2.35/2) \times [t_R(b)-t_R(a)] / [w_{50}(b)+w_{50}(a)]}$$

where t_R is the peak retention time (min), w_{50} is the peak width at half-height (min), and (a) and (b) denote peaks one and two respectively.

Method Validation

The performance of the method will be examined in terms of the linearity, repeatability, limit of detection (LOD) and limit quantification (LOQ). Linearity of the optimized method is assessed by constructing the calibration curve of average peak areas (n = 3) against the concentration of standards (at the linear range). The repeatabilities in the migration time, peak area and peak height are recognized in terms of the relative standard deviation (RSD%, n = 3). The LOD and LOQ are determined by the calibration curve along with the signal-to-noise ratio (S/N) as 3 and 10, respectively.

Results and Discussion

Effects of methanol concentration on the enantioresolution of miconazole by CD-MEKC

Methanol was optimized as organic modifier in the CD-MEKC for chiral separation of miconazole. As can be seen in the Fig. 1, enantioresolution of miconazole was obtained with 5% methanol, however the resolution is less than 1.0 (Rs < 1.0). To obtain the higher enantioresolution of miconazole, the use of acetonitrile was then investigated.

Effects of acetonitrile concentration on the enantioresolution of miconazole

Acetonitrile was optimized as organic modifier in the CD-MEKC for chiral separation of miconazole. As can be seen in the Fig. 2, the best enantioresolution of miconazole (Rs = 1.0) was obtained with 5% acetonitrile.

Enantioresolution of miconazole by HPLC method

Chiral separation of miconazole was developed in this study using high performance liquid chromatography (HPLC). The best enantioresolution of miconazole (Rs = 2.2) was achieved by the HPLC system (Fig 3) using Astec Cyclobond (25 cm × 4.6 mm × 5 μm) as chiral column, acetonitril:water (0.1% HCOOH) 20:80 as mobile phase, flow rate of 1.0 mL/min, and detection wavelength at 230 nm. The use of cyclodextrins as chiral selectors can cause inclusion bonds with molecules in the drug. These inclusion bonds can increase solubility, dissolution, stability, and molecular bioavailability. The interaction of the solute with mobile and stationary phases can be manipulated through different choices of both solvents and stationary phases. The calibration curve is linear with (r) = 0,9998 (peak 1) and (r) = 0,9937 (peak 2) on the concentration range of miconazole = 30-120 mg/L. Limit of detection (LOD) is 1,83 ppm (peak 1) and 9,26 ppm (peak 2), respectively. Limit of quantitation (LOQ) is 6,09 ppm (peak 1) and 30,86 ppm (peak 2), respectively.

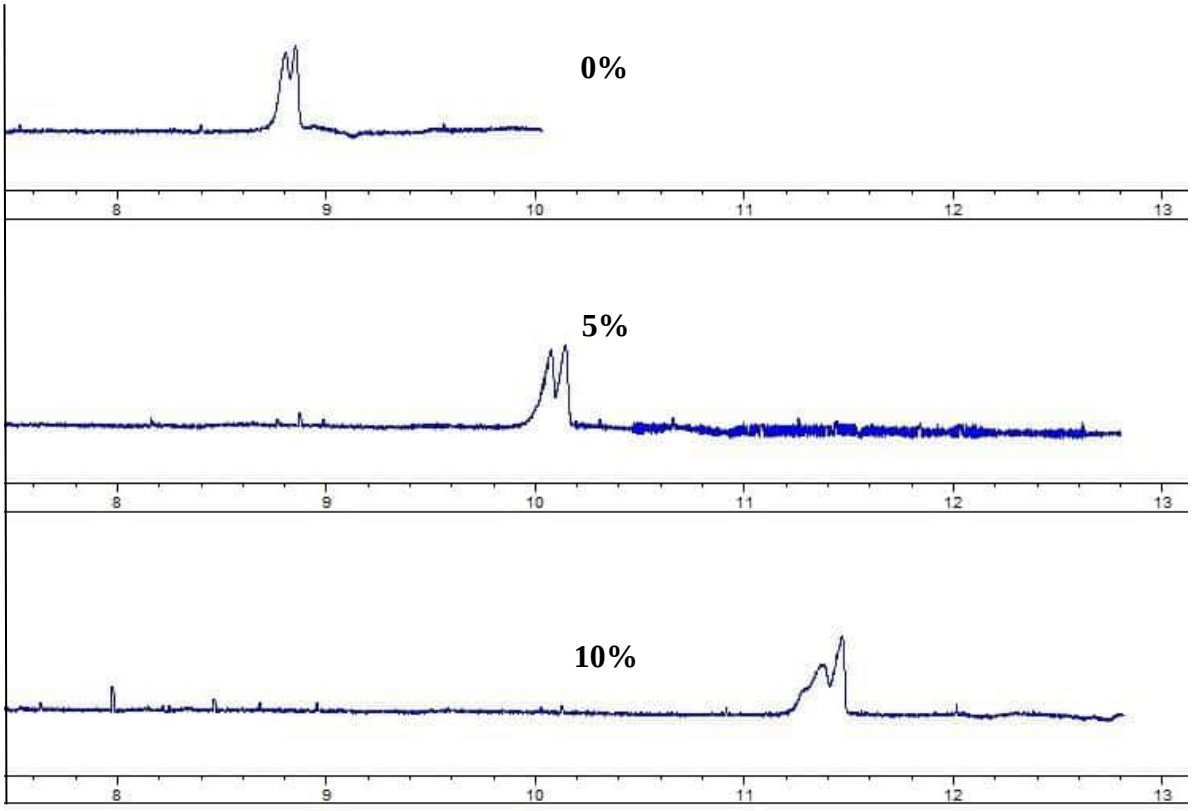

Figure 1. Effect of methanol concentration (0 – 10%) on the enantioresolution of miconazole by CD-MEKC system containing 40 mM HP-γ-CD (as chiral selector), 40 mM SDS (as surfactant), 20 mM phosphate buffer (pH = 8).

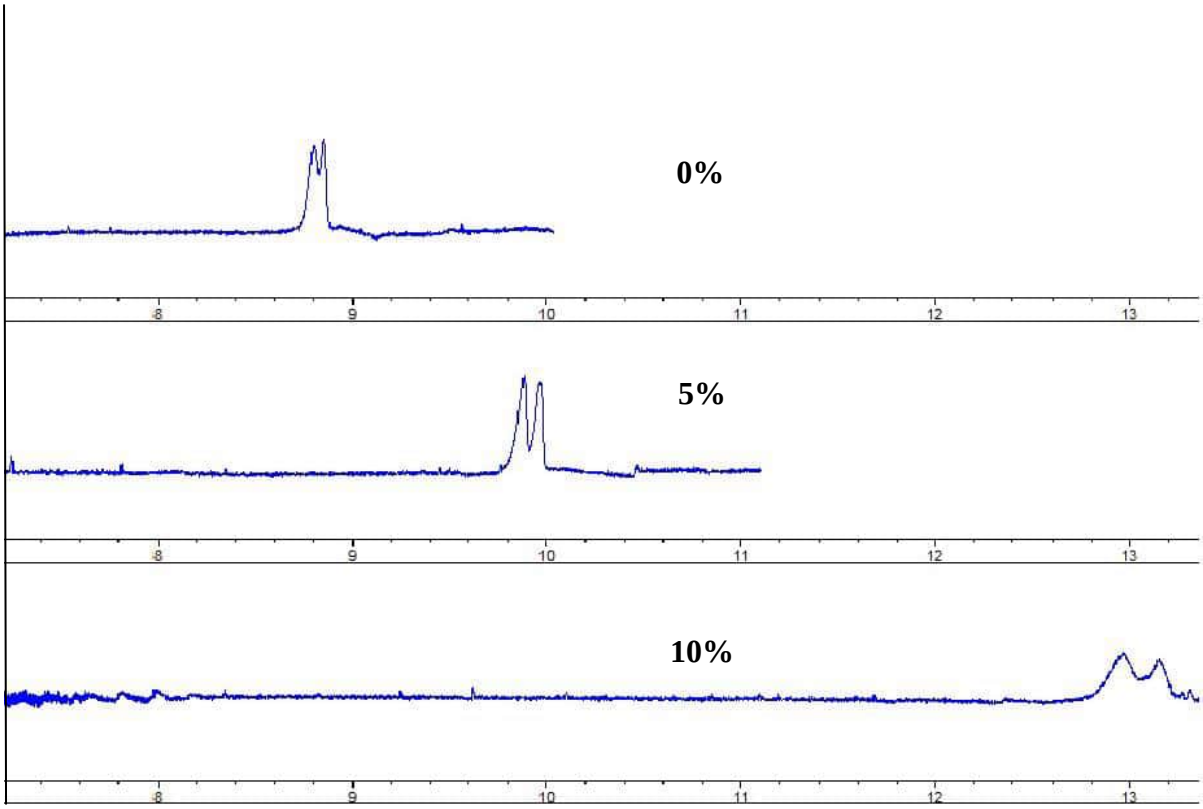

Figure 2. Effect of acetonitrile concentration (0 – 10%) on the enantioresolution of miconazole by CD-MEKC system containing 40 mM HP-γ-CD (as chiral selector), 40 mM SDS (as surfactant), 20 mM phosphate buffer (pH = 8).

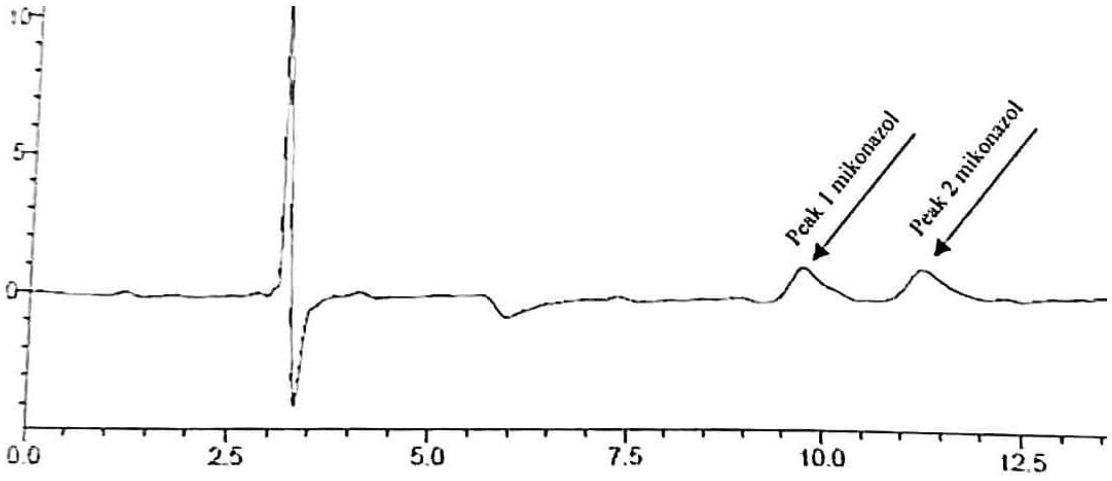

Figure 3. Enantioresolution of miconazole by HPLC method. HPLC using Astec Cyclobond (25 cm × 4.6 mm × 5 μm) as chiral column, acetonitril:water (0.1% HCOOH) 20:80 as mobile phase, flow rate of 1.0 mL/min, and detection wavelength at 230 nm.

Conclusions

Chiral separation of miconazole has been achieved with Rs = 1.0 by the CD-MEKC method using 40 mM HP-γ-CD as chiral selector, 40 mM SDS as surfactant, 20 mM phosphate buffer (pH = 8) and 5% acetonitrile. The CD-MEKC method was simple, less solvent used, and relatively short analysis time. In addition, the best enantioresolution of miconazole (Rs = 2.2) has been successfully achieved in this study by the HPLC system using Cyclobond I 2000 HP-RSP as chiral column (25 cm × 4.6 mm × 5 μm), acetonitril:water (0.1% HCOOH) 20:80 as mobile phase, flow rate of 1.0 mL/min, and detection wavelength at 230 nm. The present HPLC method has high resolution, high sensitivity and rapid analysis.

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