PROCEEDINGS PAPERS OF

1st INTERNATIONAL CONFERENCE ON CHEMISTRY, PHARMACY AND MEDICAL SCIENCES (ICCPM)

Theme: Advanced Research Development Base on Local Resources

Bengkulu, 27 – 28 November 2018

Editor: Deni Agus Triawan, S.Si., M.Sc

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FOREWORD

Assalamu’alaikum warahmatullahi wabarakaatuh and greetings.

This proceeding contains selected papers of 1st International Conference on Chemistry, Pharmacy, and Medical Sciences (ICCPM) which held on November 26-27, 2018, Santika Hotel, Bengkulu-Indonesia. The conference which was organized by the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu.

The ICCPM 2018 is attended by more than 100 participants. In terms of origin, the participants of this ICCPM are coming from 6 countries i.e. Indonesia, Japan, US, Malaysia, Thailand, and India. The conference is the first international conference organized by the Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu and is expected to be held continuously every three years.

The conference particularly encouraged the interaction of research students and developing academics with the more established academic community in an informal setting to present and to discuss new and current work. Their contributions helped to make the conference as outstanding. The papers contributed the most recent scientific knowledge known in the field of Organic Chemistry, Material Chemistry, Pharmacy, Agricultural Chemistry, and Miscellaneous topic related to chemistry.

Our deep gratitude is strongly forwarded to all individuals who took part in the conference, especially the keynote speakers, invited speakers, all the presenters and participants as well as all students and staffs who have been involved in the preparation and execution of the conference and the publication of the proceedings. Our deep gratitude also forwarded for all reviewers the manuscript for this proceedings.

These Proceedings will furnish the scientists with a good reference book. I trust also that this will be an impetus to stimulate further study and research in all these areas.

Bengkulu, 30 November 2018
General Chair of ICCPM
Prof. Dr. Morina Adfa, M.Si
Committee

1st International Conference on Chemistry, Pharmacy and Medical Sciences (ICCPM, Theme: Advanced Research Development Base on Local Resources)

Santika Hotel, 27-28 November 2018

Organized by Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu

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1. Prof. Dr. Mamoru Koketsu (Gifu University, JAPAN)
2. Prof. Dr. Yun Hin Taufiq Yap (Universiti Putra Malaysia, MALAYSIA)
3. Assoc. Prof. Dr. Agung Nugroho (Lambung Mangkurat University, INDONESIA)
4. Assoc. Prof. Dr. Sirikantjana Thongmee (Kasetsart University, THAILAND)
5. Assoc. Prof. Dr. Mohammad Abrar Alam (United State of America, USA)

Invited Speaker

1. Assoc. Prof. Dr. Mohamad Rafi (Bogor Agricultural University, INDONESIA)
2. Assoc. Prof. Dr. Noor Haida Mohd Kaus (Universiti Sains Malaysia (USM), MALAYSIA)
3. Assoc. Prof. Dr. Akhmad Sabarudin, D.Sc. (Brawijaya University, INDONESIA)
4. Assoc. Prof. Dr. Oman Zuas (Research Center for Metrology - LIPI, INDONESIA)
# Table of Contents

Foreword ............................................................................................................................ iii  
Committee ........................................................................................................................ iv  
Keynote and Invited Speaker ......................................................................................... v  
Table of Contents ............................................................................................................. vi  

1. Isolation and Structure Elucidation of Steroid from Methanol Extract of Sentang (Azadirachta excelsa (Jack.) Jacobs) Stem .............................. 1  
2. Pharmacognostic Evaluation of Sangketan Leaf (Achyranthes aspera Linn.) 5  
3. Is It Possible to Use Antihistamine as Analgesic? ................................................... 9  
4. The Effect of Benzophenone-3 Concentration on Activity of Sunscreen Cream Using Coconut Oil as Raw Material ............................................. 13  
5. Preliminary Study of Noni Fruit Extract (Morinda citrifolia L.) against Male Mice (Mus musculus L.) Swiss Webster Infected by Plasmodium berghei Anka (Study on Parasitemia Index and Histopathology of liver parenchyma cells) ................................................................. 16  
6. Tyrosinase Inhibitory Activity Test of Ethanol Extract of Papaya Leaves (Carica papaya L.) .............................................................................. 21  
7. The Effect of Ethanol Extract of Mangosteen (Garcinia mangostana L) Rind to LDL Level on NIDDM Type Rats ........................................... 27  
8. Antioxidant Activity of Jawer Kotok Leaves (Plectranthus scutellarioides (L.) R. Br.) with Various Composition and Amount of Solvents ................... 32  
9. Batch Adsorption of Toxic Synthetic Dyes onto Activated Carbon Made from Palm Fruit Shell ........................................................................... 36  
10. The Effect of Liquid Rubber Compound Concentration to Mechanic Properties of Particle Board ................................................................. 43  
12. Determination of Melamine Migration in Tableware Using High Performance Liquid Chromatography ......................................................... 52  
13. Effect of Natural Sulfur on ZnO Synthesis through Hydrothermal Method ........ 56  
14. Activity Assay and Determination Protein of Amylase Enzyme Fractionate from Amorphophallus campanulatus ...................................................... 61  
15. Synthesis and Characterization of Modified Silica/Zn as Heterogenous Catalyst ......................................................................................... 63  
16. Hydroxyapatite Synthesis from Chicken’s Egg Shell and Its Application as the Adsorbent of Methylene Blue ................................................................. 68  
17. Microencapsulation Methanol Extract of Solanum muricatum Aiton by Using Chitosan ...................................................................................... 73  
18. Production of Nanoemulsion from Moringa oleifera Extract ................................ 77  
19. The Evaluation of Society Knowledge Level about Oral Antibiotic and Its Use in Cipadung Kidul Urban Village ......................................................... 81  
20. The Influence of Medical Plant Mixture Inclusion on Performance, Carcass Quality and Organoleptic Properties in Broiler Chickens ......................... 84  
21. Geminivirus Resistance in Pepper (Capsicum annum) by The Application of Salicylic Acid ................................................................. 90
22. Ethnobotany Study of Asteraceae Family as a Traditional Medicine in Bengkulu Ethnics and as a Source of Biological Learning ................................................................. 93
23. Proximate Analysis of Seluang Batang Fish (Rasbora dusonensis) Syrup ........... 97
Determination of Melamine Migration in Tableware Using High Performance Liquid Chromatography

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Abstract. Melamine-based tableware has the advantage such lighter, stronger, and not broken easily. However, it’s not recommended for heat storage for long periods of time. Melamine migration in tableware results from the effect of food heating temperature, storage time, pH and processing. Melamine is dangerous if taken, inhaled, or absorbed through the skin. The maximum limit for melamine migration from food packaging is 30 ppm. Melamine migration studies have been carried out from the types of bowl and cup samples. In this study the sample was made in a simulation solution in the form of water and acetic acid pH 4.5 which was heated at 100 °C and 70 °C. Determination of melamine migration was carried out using HPLC method with condition: UV detection (λ 236), mobile phase acetonitrile: water (20:80), flow rate of 0.4 mL/minute, and C18 column with validation method results: correlation coefficient was 0.9993, detection limit and quantization limit were 0.00136 ppm and 0.00455 ppm, relative standard deviation 0.52923% in the acetic acid pH 4.5 and 0.21810% in water, percent of recovery of acetic acid pH 4.5 and water were 111.85% and 100.97% respectively. The highest melamine migration in the simulation sample of acetic acid pH 4.5 at 70 °C and 100 °C were 0.7210 ppm and 4.8914 ppm respectively, while in the simulation water at 70 °C and 100 °C were 0.2597 ppm and 0.7700 ppm.

Keywords: Chromatography, melamine, migration, tableware

A. Introduction

One of the most desirable tableware by the public is melamine-based tableware because it has the advantage of being diverse and attractive color design, lighter, stronger, not easily broken. However, melamine has the potential to migrate into food. The potential of melamine migration increases with increasing of contact time, contact temperature and contact surface area, the higher concentration of additive components and packaging materials, and the presence of aggressive food ingredients. The European Food Safety Authority (EFSA) states that there are three things that cause melamine contamination in food: the results of the use of siromazina pesticides, migration from food contact material and certain food additives such as acetic acid guanidino, urea and biuret [1, 2].

The FDA (Food Drug Administration) explains that when melamine is absorbed in the blood, it will interact in the kidney gland tract and will form yellow crystals which will block and damage kidney gland cells which eventually cover the kidney tract causing kidney malfunction [3]. Exposure to melamine in adults as measured by urine melamine levels which are also associated with gout and calcium urolithiasis [3]. Because of the uncertainty of the safety intake of melamine doses in humans, the World Health Organization (WHO) and the Food and Drug Administration (FDA) recommend daily intake that can be tolerated from 0.5 mg/Kg BW/day to 0.2 mg/Kg BW/day and from 0.63 mg/Kg BW/day to 0.063 mg/Kg BW/day [4,5].

Provisions regarding the maximum limit of melamine migration from food packaging into foodstuffs have been regulated in the Regulation from National Agency and Food and Drug Control Number Regulation number HK.03.1.23.07.11.6664 in 2011 stated that food packaging for melamine monomers with a maximum of 30 µg/L [6]. This study aims to determine the migration of melamine in tableware using the High Performance Liquid Chromatography method.

B. Results and Discussion

2.1 Optimization of analytical parameters

All compounds were successfully separated using the procedures (OSD GL-Sciences C18, 150 mm x 4.6 mm x 5µm, 20 µL sample volume, a mobile phase composed of acetonitrile and water (20 : 80) at a flow rate 0.4 ml/minute, ambient temperature, detection wavelength of 236 nm) and several chromatographic parameters, such as peak asymmetry, peak resolution, number of theoretical plates, capacity factor, repeatability retention time, were calculated from experimental data. Results are given in Table 1 and obtained values are in good agreement with limits set by validation authorities (ICH; European Pharmacopoeia) The repeatability (Table 1) of the method was checked by six injections of melamine
standard solution and was expressed as the relative standard deviation values (RSD%). The chromatogram in Fig. 1 was obtained using the HPLC method for separation of mentioned substances in the standard melamine solution.

2.2 Method Validation

The validation parameters included linearity, precision and accuracy. The results are described in Table 2. Linearity was established with a series of working solutions prepared by dilution of the melamine stock solution in mobile phase to the final concentrations corresponded to 2, 4, 6, 8, 10, 12, 14 µg/L. The calibration graphs thus involved six experimental points for each compound and solutions were injected in triplicate.

Table 1. Evaluation of separation process

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Criteria</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak asymmetry</td>
<td>0.8 &lt; T &lt; 2</td>
<td>1.004</td>
</tr>
<tr>
<td>Peak resolution</td>
<td>R &gt; 1.5</td>
<td>4.119</td>
</tr>
<tr>
<td>Number of theoretical plates</td>
<td>N &gt; 1,500</td>
<td>1,505</td>
</tr>
<tr>
<td>Capacity factor</td>
<td>2 &lt; k’ &lt; 10</td>
<td>2.666</td>
</tr>
<tr>
<td>Repeatability of retention time (%)³</td>
<td>RSD &lt; 1</td>
<td>0.089</td>
</tr>
</tbody>
</table>

³ RSD for repeated injections of melamine standard solution (n = 6)

Fig. 1. HPLC chromatogram of melamine standard solution using OSD GL-Sciences C18, 150 mm x 4. 6 mm x 5µm, mobile phase acetonitrile and water (20 : 80), flow rate 0.4 mL/minute.

The correlation coefficient values was 0.9987. To validate the precision (Table 2) of the method, six sample of solution were prepared from water and acetic acid pH 4.5 as sample simulation, both formulation were analyzed consecutively; three injections for each preparation were used for evaluation. The results were expressed as RSD values and obtained results were 0.4743 for acetic acid pH 4.5 and 0.1951 for water. The accuracy of the method was carried out to measure the sample simulation fortified with a known quantity of the analytes or spiked-placebo (addition with 2 ppm melamine standard solution) with only one concentration level. Obtained values of the recoveries (and RSD) were 99.83% (1.46%) for acetic acid pH 4.5 and 100.37% (1.01%) for water.

2.3 Determination of melamine migration

The developed method was then applied for the determination of melamine migration in sample (3 types of bowl and cup) using acetic acid pH 4.5 and water as simulation sample at temperature of 70 °C and 100 °C, the obtained results were given in Table 3.
### Table 3. Determination of melamine migration

<table>
<thead>
<tr>
<th>Simulation sample</th>
<th>Sample code*</th>
<th>Determination of melamine (µg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>70 °C</td>
</tr>
<tr>
<td>Water</td>
<td>B1</td>
<td>0.1767 ± 0.0001</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>0.2092 ± 0.0006</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>0.2039 ± 0.0005</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>0.2597 ± 0.0016</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>0.1908 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>0.1870 ± 0.0002</td>
</tr>
<tr>
<td>Acetic acid pH 4.5</td>
<td>B1</td>
<td>0.2094 ± 0.0006</td>
</tr>
<tr>
<td></td>
<td>B2</td>
<td>0.1901 ± 0.0002</td>
</tr>
<tr>
<td></td>
<td>B3</td>
<td>0.2969 ± 0.0024</td>
</tr>
<tr>
<td></td>
<td>C1</td>
<td>0.5434 ± 0.0073</td>
</tr>
<tr>
<td></td>
<td>C2</td>
<td>0.7210 ± 0.0109</td>
</tr>
<tr>
<td></td>
<td>C3</td>
<td>0.3917 ± 0.0043</td>
</tr>
</tbody>
</table>

* B = code for bowl; C = code for cup

### C. Conclusion

The highest melamine migration in the simulation sample of acetic acid pH 4.5 at 70 °C and 100 °C were 0.7210 µg/L and 4.8914 µg/L respectively, while in the simulation water at 70 °C and 100 °C were 0.2597 µg/L and 0.7700 µg/L.

### D. Experimental Section

#### 4.1 Materials

Working standards of melamine was provided by Merck (Darmstadt, Germany). HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany), acetic acid was obtained from Merck (Darmstadt, Germany), the deionised water from Ikapharmindo Putramas (Jakarta, Indonesia). Analyses were performed using HPLC Shimadzu LC-20 AT, column C18 OSD GL-Sciences (150 mm x 4.6 mm, 5 µm).

#### 4.2 Chromatographic conditions

The analyte (melamine) were successfully separated at about 3.5 min by isocratic elution on the column (OSD GL-Sciences C18, 150 mm x 4.6 mm x 5 µm), with a mobile phase composed of acetonitrile and water (20 : 80) at flow rate 0.4 mL/minute, detection was observed at wavelength 236 nm.

#### 4.3 Standard preparation

Stock and standard solution of melamine were prepared by dissolution in mobile phase with final concentration were 2, 4, 6, 8, 10, 12, 14 µg/L.

#### 4.4 Sample collection and preparation

The sample used in this experiment were bowl and cup with melamine ware label. All samples were collected from Ujung Berung Market, Bandung.

The sample were made by preparing two simulation solutions. The simulation used were acetic acid solution pH 4.5 and water. Each simulation solution was heated with temperatures 70 °C and 100 °C. Then put it in a melamine cup and bowl. The simulation solution with a temperature of 70 °C was allowed to stand for 2 hours, while the simulation solution with a temperature of 100 °C was allowed to stand for 15 minutes, then the solution was filtered using a membrane filter 0.45 µm and ready to be injected into the HPLC.

### E. References

2. European Food Safety Authority, (2010). Scientific opinion on melamine in food and feed, EFSA panel on contaminants in the food chain (CONTAM) and EFSA panel on food contact materials, enzymes, flavourings and processing aids (CEF), *EFSA Journal*, 8(4), 1573.
