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INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

Surabaya, July 21 - 22, 2010

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38th Meeting of National Working Group on Indonesian Medicinal Plant

ISBN : 978-602-96839-1-2
978-602-96839-3-6

Proceedings

International Conference On Medicinal Plants

The Future of Medicinal Plants:
From Plant to Medicine

Surabaya, 21-22 July 2010

Organized by

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PREFACE

The International Conference on Medicinal Plants in occasion of the 38th Meeting of National Working Group on Medicinal Plant was held on the campus of Widya Mandala Catholic University in Surabaya during 21-22 July 2010. Over 300 participants had many fruitful discussions and exchanges that contributed to the success of conference. The present volume Proceedings (Volume 2) includes the papers presented at the conference and continues where Volume 1 leaves off.

The 192 abstracts that were presented on two days formed the heart of the conference and provided ample opportunity for discussion. Of the total number of presented abstracts, 63 of these are included in the Volume 1 and 58 in this proceedings volume. Both of the Conference Proceedings cover all aspects on key issues related to medicinal uses of plants, their active ingredients and pharmacological effects, production and cultivation of medicinal plants.

We appreciate the contribution of the participants and on behalf of all the conference participants we would like to express our sincere thanks to plenary speakers, Dr. Mona Tawab, Prof. Henk van Wilgenburg, Prof. Tohru Mitsunaga, Prof. De-An Guo, dr. Arijanto Jonosewojo, SpPD FINASIM, Dr. Bambang Prayogo, Mr. Jimmy Sidharta, Ir. Dwi Mayasari Tjahjono, S.Pd, Dipl. Cidesco,Dipl. Cibtac , and everybody who helped to make conference success and especially to our sponsors

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May you all be richly rewarded by the LORD.

All in all, the Conference was very successful. The plenary lectures and the progress and special reports bridged the gap between the different fields of the development of medicinal plants, making it possible for non-experts in a given area to gain insight into new areas. Also, included among the speakers were several young scientists, namely, students, who brought new perspectives to their fields. I hope this proceedings will promote the interdisciplinary exchange of knowledge and ideas in medicinal plant and related industries.

Dr.phil.nat. Elisabeth Catherina Widjajakusuma
Conference Chairman

ISBN : 978-602-96839-3-6(vol 2)

CONTENT

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DETECTION OF CHLORAMPHENICOL RESIDUE IN SHRIMP (*PENAEUS MONODON*) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract : In spite of its potential toxicity, chloramphenicol at its therapeutic dose is used for the treatment of various infections in human. Since chloramphenicol is used as a preservative in food producing animals and animal feed products, many countries have made a strict regulation to control the residual content of chloramphenicol in meat, including shrimp meat (*Penaeus monodon*). The aim of this study was to develop a method for the detection of chloramphenicol in shrimp meat at the part per billion (ppb) level. The shrimp meat was homogenized in saline buffer, extracted using chloroform, evaporated and then reconstituted in the mobile phase. The resulting solution was injected onto an HPLC-C_{18} column and detected at 265 nm. A mixture of methanol: water (30:70, v/v) was used as a mobile phase. A linear curve of the standard solution ranging between 0.10 and 1.0 µg/ml was constructed. The overall recovery of analyte was found to be 82.77 (± 3.17%). The limit of quantitation (LOQ) and the limit of detection (LOD) of chloramphenicol were 0.28 ng per g of shrimp (0.28 ppb) and 0.09 ppb, respectively.

Keywords: chloramphenicol, shrimp meat, HPLC

INTRODUCTION

Chloramphenicol is a broad spectrum antibiotic that was first isolated from *Streptomyces venezuelae* and it has very effective antibacterial properties, which interferes with protein synthesis of many gram-negative and gram-positive bacteria. Miscellaneous toxic effects are due to the dichloride carbon alpha to the carbonyl group. This carbon readily undergoes substitution with nucleophiles such as those found on proteins as seen in the Figure 1.

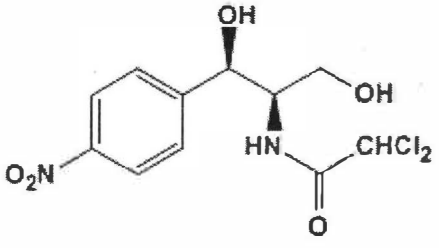

Figure 1. Structure of Chloramphenicol

The main potential human toxicity is depression of red blood cell production in bone marrow leading to aplastic anemia (Neuhaus *et al.*, 2002). Because of the unpredictable effects of dose on different patient populations, many country regulations prohibit its use in food producing animals and animal feed products.

Analytical methods for assaying chloramphenicol in shrimp have been available for a number of years, using many methods. The Gas Chromatography (GC) - Electron Capture Detector (ECD) method has an effective detection limit of 5 parts per billion (ppb) and relies on chromatographic retention time for identification. Liquid Chromatography (LC) using ultra violet (UV) detection was also commonly used which

gave detection and quantitation limits at 5 and 10 ppb respectively in aquaculture tissue. The GC using mass spectrometry (MS) detection was used with detection limits at about 1 ppb. Liquid Chromatography (LC) using MS detection was also commonly used which gave detection and quantitation limits at 0.08 and 0.3 ppb respectively. At least two enzyme linked immunosorbent assay (ELISA) kits have recently been developed which claim to be able to detect chloramphenicol in seafood tissue low ppb region, but the results using these kits have not been published (Stuart, 2002). However modifications and new methods should lower the detection limit to at or below 1 ppb.

A common approach to the analysis of chloramphenicol in seafood tissues was first cleanup utilizing liquid/liquid extraction and solid phase extraction followed by derivatization to form volatile derivatives, and analyzed by GC-ECD. The other method was pulverized with dry ice, extracted with ethyl acetate, evaporated with N2, treated with hexane/aqueous NaCl, extracted back into ethyl acetate, dissolved into methanol-water after evaporation, and injected into an LC/MS. It showed that the preparation sample was difficult and spent a lot of reagent. However, this study was done to develop a simple method for the detection of chloramphenicol in shrimp meat at the part per billion (ppb) level by High Performance Liquid Chromatography using UV detection.

MATERIAL AND METHODS

Reagents

Chloroform (pro analysis), Sodium diphosphat (pro analysis), disodium phosphate (pro analysis), methanol (HPLC Grade), Water (aqua pro injection), and Chloramphenicol (pharmaceutical grade).

Standard Solution

A chloramphenicol standard stock solution of 1,0 mg/mL was prepared by dissolving 100 mg chloramphenicol in 100 mL of methanol. The working solution of 5 µg/mL was made by diluting a stock solution with methanol : water (30 : 70, v/v) as the mobile phase.

HPLC Condition

High Performance Liquid Chromatography analyses were performed on a C18 Licrosorb column (100 x 2 mm i.d.) (E-Merck, USA) using an Hitachi series liquid chromatograph equipped with a isocratic system. Mobile phase was methanol: water (30:70; v/v). The flow rate was set at 1 mL/min and the injection volume was 20 µL.

Sample Preparation

Three hundred grams (300g) of headless, peeled and defrozen shrimp meat was homogenized in saline buffer and extracted using chloroform. The extract dried with waterbath at 45±5°C and diluted into a sufficient volume using mobile phase.The extract was then passed through a 0.2µm membrane filter and ready for analysis.

RESULTS AND DISCUSSION

Sample Preparation:

A major goal for the method development in this study is to avoid using the labor intensive and reagent-consuming procedures as in literatures. In current work, the shrimp meat were destructed with saline buffer, followed by extraction the chloramphenicol with chloroform, which is necessary for chromatographic separation. Thus, the extract was evaporated and then reconstituted in mobile phase, before be taken for HPLC analysis.

Method Performance

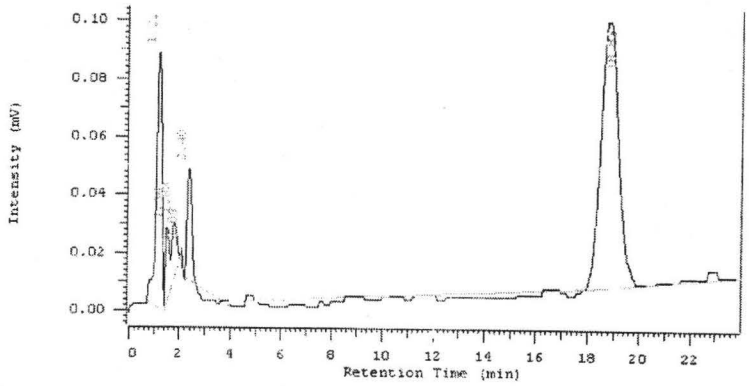

Figure 2. Chromatograms of Chloramphenicol (1 µg/mL) in shrimp meat

Figure 2 shows representative chloramphenicol in shrimp meat chromatograms. It showed that the mobile phase methanol: water (30:70, v/v) was selected to achieve maximum separation and sensitivity. Under these conditions the retention times values of chloramphenicol was 18 minutes and detected at 265 nm (figure 3).

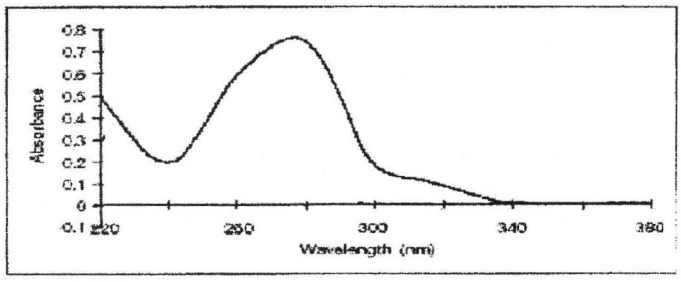

Figure 3. Absorption spectrum of chloramphenicol from 200 – 280 nm

All the traces can be well separated. It should also be noted that the chloramphenicol peak width (at 10% above baseline) is still narrow.

A representative calibration curve from 3 replicate standards interday prepared in mobile phase was shown in Figure 4. Good linearity from 0.1 to 1.0 µg/mL with correlation coefficient of R^2= 0.9969 – 0.9983 was obtained.

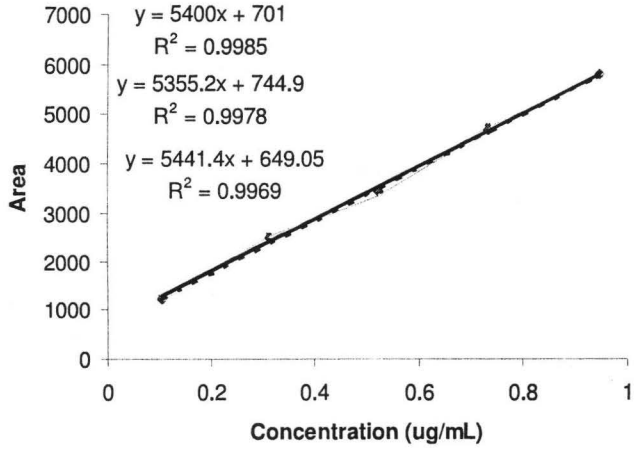

Figure 4. Calibration of three replicate chloramphenicol standard solution interday

The analyte concentration in a typical matrix blank was determined by spiking the chloramphenicol working solution (5µg/mL) into the matrix blank before sample preparation. The determination was reported in Table 1.

Table 1. Accuracy and Precision Data of Chloramphenicol in Shrimp Meat

Replication	Recovery (%)
1	84.4
2	82.8
3	86.7
4	82.6
5	80.9
6	79.2
	82.77 %(±3.17%)

It ranged from 79.2 for the lowest level of recovery to 86.7 for the highest level.

According to the Symposium on Harmonization of Quality Assurance Systems for Analytical Laboratories in Budapest, Hungary, 4–5 November 1999, (Thompson, 2002), the detection limit as estimated in method development may not be identical in concept or numerical value to one used to characterize a complete analytical method. It is accordingly recommended that for method validation, the precision estimate used (S_0) should be based on at least 6 independent complete determinations of analyte concentration in a typical matrix blank or low-level material, with no sensoring of zero or negative results, and the approximate detection limit calculated as $3S_0$. The limit of quantitation (LOQ) and the Limit of Detection (LOD) of chloramphenicol were 0.28 ng per g of shrimp (0.28 ppb) and 0.09 ppb, respectively.

Confirmation

Chloramphenicol is identified by chromatographic retention time over the course of the analysis sets in as many weeks, the retention times were predictable and consistent. For all analyses, the retention time ranged from a high of 18.90 minute to a low of 18.35 minute.

CONCLUSIONS

The method presented here is economical of both time and material. It simultaneously provides reliable determination and confirmation of chloramphenicol in shrimp meat which is useful in a regulatory situation.

ACKNOWLEDGEMENTS

We would like to thanks to Faculty of Pharmacy, Widya Mandala Catholic University for supporting the research facilities and financing.

REFERENCES

Neuhaus B.K., Hurlbut J. A., Hammack W., 2002, LC/MS/MS Analysis of Chloramphenicol in Shrimp, *Laboratory Information Bulletin*, **4290**, 1-18.

Stuart J.S., Rupp H. S., Hurlbut J. A., 2002, LC/MS/MS Analysis of Chloramphenicol in Crawfish Meat, FDA/ORA/DFS, LIB#4303, 1-10

Thompson M., Ellison S. L. R., Wood R., 2002, Harmonized Guidelines for Single Laboratory Validation of Methods of Analysis, *Pure Appl. Chem*, **74**, 835-855