UNIVERSITI PUTRA MALAYSIA

PREPARATION OF 3[(3-CARBOXYPHENYL) METHYLENE]-HYDRAZINECARBOXAMIDE FOR IMMUNOASSAY OF NITROFURAN ANTIBIOTICS

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MASTER OF SCIENCE
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2011

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By

AZIMA AZMI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

June 2011

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

PREPARATION OF 3[(3-CARBOXYPHENYL) METHYLENE]-HYDRAZINECARBOXAMIDE FOR IMMUNOASSAY OF NITROFURAN ANTIBIOTICS

By

AZIMA BINTI AZMI

June 2011

Chairman: Professor Faujan B. Ahmad @ Haji Amat, PhD

Faculty: Science

Currently, analysis of Semicarbazide (SEM) is being carried out using LC-MS/MS instrumentation. It is a need to developed rapid immuno based test kit. We described the synthesis of modified SEM hapten has been carried out through chemical reaction.

Polyclonal antibody (pAb) was produced to detect Semicarbazide (SEM), metabolite as a marker residue of nitofurazone in animal food production. A carboxyphenyl derivative of SEM was synthesized following derivatisation of 4-carboxylbenzaldehyde (4-CBA).

The modified SEM hapten was characterized using thin layer chromatography (TLC) with Rf value of 0.53 in crystalline form. The fourier transform infra-red (FTIR) spectrum exhibits the peaks at Vmax 3584cm-1 for C=O (carboxylic group). The melting point for

the synthesized hapten is 220-223oC.

After purification of hapten, the newly developed hapten was then conjugated to keyhole limpet hemocyanin (KLH) as immunogen using 1-ethtyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) method for conjugation purpose. This immunogen (SEM-KLH) were used to immunize two New Zealand white rabbit to produce polyclonal antibody against newly developed hapten. Using direct enzyme linked immunoassay (ELISA) method, titer determination of the developed antibody of 0.01mg/ml was obtained.

For conjugation efficiency electrophoresis gel SDS-PAGE were carried out using 10-7% of resolving gel. This is to identify protein band according to commercial protein marker. No band occur for both of this resolving gel concentration but band for KLH occur at 7% resolving gel but no band for conjugated hapten-protein.

Another confirmation of the developed antibody efficiency test is done using surface plasmon resonance which a well is known as biosensor instrument. According to the result, limit of detection for this antibody is up to 0.1 x 10-7 mg/ml compared to ELISA method

Abstrak tesis dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains.

PREPARATION OF 3[(3-CARBOXYPHENYL) METHYLENE]-HYDRAZINECARBOXAMIDE FOR IMMUNOASSAY OF NITROFURAN ANTIBIOTICS

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Jun 2011

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Pada masa kini, analisis bagi pengenalpastian Semikarbazida (SEM) dilakukan dengan menggunakan peralatan LC-MS/MS. Pembangunan kit pengesan berasaskan immunoasai adalah sangat diperlukan. Disini kami menerangkan sintesis bagi pengubahsuaian hapten SEM melalui kaedah tindak balas kimia.

Antibodi poliklonal telah dihasilkan untuk mengesan SEM, dimana ianya merupakan metabolit yang digunakan sebagai penanda sisa baki nitrofurazon didalam penghasilan makanan binatang ternakan. Derivatif karboksilfenil bagi SEM dihasilkan melalui terbitan dari 4-karboksibenzaldehid (4-CBA).

Hapten SEM yang telah diubahsuai melalui proses pencirian yang menggunakan

kromatografi lapisan tipis (TLC) denga nilai Rf 0.53 didalam bentuk kristal. Bagi Fourier Infra Merah (FTIR) pula menunjukkan puncak Vmax 3584cm-1 bagi C=O (kumpulan karboksil). Takat lebur yang dicatatkan pula ialah 220-223oC.

Selepas penulenan hapten, hapten yg baru dibangunkan kemudiannya dikonjugat kepada *Keyhole Limpet Hemocyanin* (KLH) yang bertindak sebagai immunogen menggunakan kaedah 1-ethtyl-3-(dimethylaminopropyl) carbodiimide hydrochloride (EDC) bagi tujuan konjugasi. Immunogen (SEM-KLH) digunakan untuk immunisasi terhadap dua ekor arnab putih New Zealand bagi menghasilkan antibodi poliklonal terhadap hapten yang baru dibangunkan. Penentuan titer bagi antibodi yang terhasil adalah 0.01mg/ml.

Bagi pengenalpastian keberkesanan konjugasi, gel elektroforesis SDS-PAGE telah dijalankan menggunakan 10-7% gel pemisah. Ini bagi membuat perbandingan dengan protin penanda komersil. Tiada jalur yang wujud bagi kedua-dua kepekatan gel namun pada kepekatan 7% jalur protin bagi KLH telah kelihatan tetapi masih tiada jalur bagi konjugasi hapten tersebut.

Satu lagi ujian pengesahan keberkesanan antibodi yang dibangunkan ini telah dijalankan menggunakan *surface plasmon resonance* iaitu alat yang juga dikenalpasti sebagai alat biosensor. Berdasarkan keputusan yang diperolehi, didapati, antibodi ini dapat mengesan sehingga 0.1 x 10-7 mg/ml berbanding kaedah ELISA.

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I certify that an Examination Committee has met on 3rd June 2011 to conduct the final examination of Azima Binti Azmi on her Master of Science thesis entitled _Preparation of 3[(3-Carboxyphenyl) Methylene]-Hydrazinecarboxamide for Immunoassay of Nitrofuran Antibiotics' in accordance with University Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be rewarded the degree of Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or any other institution.

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AZIMA BINTI AZMI

DATE: 3 June 2011

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