

**DETECTION OF BETA-AGONIST RESIDUES IN MEAT
USING ENZYME LINKED IMMUNOSORBENT ASSAY
AND GAS CHROMATOGRAPHY MASS SPECTROMETRY**

By

P. JEYALETCHUMI A/P S. PONNIAH

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Masters of Science
November 2003**

DEDICATION

Dedicated to my mother, Retnam Thambipillai,

my husband, Kanesan Sathianathan

my sons, Thivagar and Gajendra

Abstract of thesis submitted to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Master of Science

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Chairman : Associate Professor Sharifah Kharidah bt. Syed Muhammad,Ph.D.

Faculty : Food Science and Biotechnology

The prevalence, type and concentration of beta-agonist residues in the liver and meat of three types of livestock animals i.e. goats, cattle and swine were studied using Gas Chromatography-Mass Spectrometry. Beta-agonist residues were only detected in swine with a prevalence of 16.6% in meat and 20% in liver sampled. The concentration of beta-agonist residues in the positive samples ranged between 1ng/g to 9ng/g. The performance of the multi-residue analysis method used was assessed through recovery studies and found to be varied among the beta-agonists wherein terbutaline showed the highest recovery values (78-83%) whereas salbutamol showed the lowest recovery values (22% -31%). The coefficient of variation (C.V.) had values between 1-12% which indicate acceptable variation for the method.

In the second phase of this study, three ELISA test-kits, i.e. Randox ELISA beta-agonist test kit, Euro-Diagnostica beta-agonist test kit and Ridascreen beta-agonist test kit were evaluated for screening of meat and liver for beta-agonist residues in fortified and field incurred tissue samples. It was found that the Randox beta-agonist test kit was more suitable as a screening tool due to its accuracy, ease of use and lower cost. The test-kit was able to detect beta-agonists at the minimum level of detection as claimed by the suppliers. The performance of the method as assessed through recovery rates of the beta-agonists in fortified samples was satisfactory with a low coefficient of variation (1-3%). Reproducibility, as measured through the coefficient of correlation was also satisfactory. For field-incurred positive samples, the test kit showed a sensitivity of 100% and a low rate of false positives (less than 10%) for goat and cow tissues. However a high rates of apparent false positives (50%-65%) was obtained for tissues of swine.

The third phase of the study evaluated the cross-reactivities of the antibodies within the three test kits to other veterinary drugs normally administered to swine. It was found that sulfachlorpyridazine; sulfamethaxine; penicillin G and amantidine cross-reacted with all three ELISA test kits to give a positive response.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi syarat keperluan untuk Ijazah Master Sains

**PENGENALPASTIAN KANDUNGAN RESIDU BETA-AGONIST
DALAM DAGING MENGGUNAKAN KAEDAH ‘ENZYME-LINKED
IMMUNOSORBENT ASSAY’ DAN ‘GAS CHROMATOGRAPHY-
MASS SPECTROMETRY’**

oleh

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Pengerusi : Profesor Madya Sharifah Kharidah bt. Syed Muhammad, Ph.D.

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Kehadiran, jenis dan kandungan residu beta-agonist di dalam daging dan hati tiga jenis haiwan ternakan iaitu lembu, kambing dan khinzir telah dikaji dengan menggunakan peralatan ‘Gas Chromatography Mass Spectrometry’. Residu beta-agonist cuma dapat dikesan pada tisu khinzir, iaitu sebanyak 16.6% daripada daging dan 20% daripada hati yang disampel. Kandungan beta-agonist di dalam sampel positif berada dalam julat 1ng/g hingga 9ng/g. Keberkesanan kaedah multi-residu yang digunakan telah diuji menggunakan kajian ‘recovery’ dan didapati berbeza antara beta-agonist yang berlainan, iaitu terbutalin menunjukkan nilai ‘recovery’ tertinggi (78 –83%) sementara salbutamol menunjukkan nilai ‘recovery’ terendah (22-31%). Nilai koefisi variasi berada di antara 1-12% yang menunjukkan variasi yang boleh diterima bagi kaedah yang digunakan.

Dalam fasa kedua kajian ini, tiga peralatan ujian ELISA, iaitu peralatan ujian Randox; peralatan ujian Euro-Diagnostica dan peralatan ujian Ridascreen telah dibandingkan bagi pengskrinan residu beta-agonist dalam sampel tisu yang difortifikasi dan 'field-incurred'. Didapati bahawa peralatan ujian Randox paling sesuai sebagai alat pengskrinan kerana ia lebih jitu, mudah digunakan dan murah. Peralatan ujian ini dapat mengesan residu beta-agonist pada tahap pengesanan minima seperti yang diakui oleh pembekal. Keberkesanan kaedah seperti yang dinilai melalui kadar 'recovery' beta-agonist yang difortifikasi didapati memuaskan dengan kadar koefisi variasi yang rendah (1-3%). 'Reproducibility' yang diukur melalui koefisi korrelasi juga didapati memuaskan. Bagi sampel positif yang 'field-incurred', ujian peralatan menunjukkan sensitiviti 100% dan kadar positif palsu yang rendah (kurang dari 10%) untuk tisu kambing dan lembu. Bagaimanapun, kadar positif palsu yang tinggi (50-65%) didapati bagi tisu khinzir.

Fasa ketiga kajian ini telah menilai 'cross-reactivity' di antara antibodi di dalam ketiga-tiga peralatan ujian tersebut dengan dadah veterinar lain yang biasanya diberi kepada haiwan khinzir. 'Sulfachlorpyridazine', 'sulfamethazine', penicillin G dan amantidin didapati saling bertindak dengan antibodi didalam ketiga-tiga peralatan ujian ELISA untuk memberi respon positif.

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I certify that an Examination Committee have met on November 8, 2003, to conduct the final examination of P.Jeyaletchumi a/p S.Ponniah on her Master of Science thesis entitled “ Detection of Beta-agonist Residues in Meat Using Enzyme-linked Immunosorbent Assay and Gas Chromatography-Mass Spectrometry” in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Putra Malaysia (Higher Degree) Regulations 1981. The Committee recommended that the candidate be awarded the relevant degree. The Committee Members for the candidate are as follows :

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DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any degree at UPM or other institutions.

(P. Jeyaletchumi a/p S. Ponniah)

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