UNIVERSITI PUTRA MALAYSIA

DEVELOPMENT, OPTIMIZATION AND VALIDATION OF LC-MS/MS METHOD FOR MULTI-MYCOTOXIN DETECTION IN CEREALS

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By

FARHANG SOLEIMANY

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

April 2011

DEDICATION

To my beloved wife

Anosheh

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirements for the degree of Doctor of Philosophy

DEVELOPMENT, OPTIMIZATION AND VALIDATION OF LC-MS/MS METHOD FOR MULTI-MYCOTOXIN DETECTION IN CEREALS

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Chairman : Professor Jinap Selamat, PhD

Faculty : Food Science and Technology

Mycotoxins are fungal natural metabolites that have a wide range of toxic effects. Among hundreds of mycotoxins, aflatoxins (AFs) (AFB_1, AFB_2, AFG_1, and AFG_2), ochratoxin A (OTA), zearalenone (ZEA), deoxynivalenol (DON), fumonisins (FB_1 and FB_2,), T2 and HT2-toxins are the major health concerns for humans and domestic animals. First, an HPLC method has been developed to investigate the separation of mycotoxins in liquid chromatography. Two derivatization systems, photochemical and chemical methods were applied for derivatization of AFB_1 and AFG_1, as well as FB_1 and FB_2, respectively. Then, a LC-MS/MS method has been developed by evaluating the effect of LC column (50 and 150 mm), organic modifier (methanol and acetonitrile) ionization process (ESI, APCI) and ionization mode (positive and negative) on separation and determination of mycotoxins. Then the developed

method was optimized for simultaneous determination of the 11 mycotoxins. Response surface methodology (RSM) was used to optimize the LC conditions. The effect of organic solvent percentage at the beginning (0-20%) and end (75-95%) of gradient mobile phase, acid concentration in aqueous phase (0-1%), and flow rate (100-300 µl/min) have been investigated for optimization of LC responses peak area and signal to noise ratio (S/N).

The optimized responses obtained using following conditions: organic solvent of 5% at start and 95% at the end of gradient mobile phase, 0.1% acid concentration, and 250 µl/min flow rate. In addition, best sample preparation procedure have been selected by evaluating the effects of two different common types of solvent extraction methods (one step and two step extraction) and four types of clean-up methods including Oasis HLB, MycoSep, immunoaffinity column (IAC) and no clean-up on mycotoxins recoveries. The results of the study showed that the best recoveries (79-109%) for all mycotoxins would be obtained by using one step extraction with no clean up. Finally, the optimized LC-MS/MS method was validated by measuring the selectivity, sensitivity, linearity, accuracy and precision. Limit of Detection (LOD) for AFB_1, AFB_2, AFG_1, AFG_2, DON, T2-Toxin, HT2-Toxin, FB_1, FB_2, OTA and ZEA was 0.05, 0.25, 0.05, 0.5, 5, 2, 2, 10, 10, 0.01, and 0.1, whereas the Limit of Quantification (LOQ) was 0.1, 0.5, 0.1, 1, 10, 4, 4, 20, 20, 0.02, and 0.2 ppb, respectively. Finally, the optimized and validated LC-MS/MS method was applied on real cereal samples (rice, barley, oat, wheat and maize) collected from Malaysian markets. The results showed applicability of the aforementioned method for being

used as fast routine method with high accuracy and precision for simultaneous determination of mycotoxins in cereal.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagi memenuhi keperluan untuk ijazah Doktor Falsafah

PEMBANGUNAN, OPTIMASI DAN VALIDASI KAEDAH LC-MS/MS UNTUK PENGESANAN MULTI-MIKOTOKSIN DALAM BIJIRIN

Oleh

FARHANG SOLEIMANY

April 2011

Pengerusi : Profesor Jinap Selamat, PhD

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Mikotoksin merupakan metabolit semulajadi cendawan yang mempunyai pelbagai kesan beracun. Di antara ratusan mikotoksin, aflatoksin (AFS) (AFB_1, AFB_2, AFG_1, dan AFG_2), ochratoxin A (OTA), zearalenon (Zea), deoxynivalenol (DON), fumonisins (FB_1 dan FB_2), T2 dan HT2-racun masalah kesihatan utama bagi manusia dan haiwan domestik sahaja. Pertama, kaedah KCKT telah dibangunkan untuk menyiasat pemisahan mikotoksin dalam kromatografi cair. Dua derivatisasi sistem, fotokimia dan kaedah kimia digunakan untuk derivatisasi dari AFB_1 dan AFG_1, serta FB_1 dan FB_2, masing-masing. Kemudian, sebuah kaedah LC-MS/MS telah dibangunkan oleh menilai kesan daripada medan LC (50 dan 150 mm), modifier organik (metanol dan asetonitril) proses pengionan (ESI, APCI) dan mod pengionan (positif dan negatif) pada pemisahan dan penentuan mikotoksin. Kemudian kaedah

yang dibangunkan adalah dioptimumkan untuk penentuan serentak dari 11 mikotoksin. Respon permukaan metodologi (RSM) digunakan untuk mengoptimumkan keadaan LC. Pengaruh peratusan pelarut organik di bermula (0-20%) dan akhir (75-95%) dari kecerunan fasa gerak, konsentrasi asid dalam fasa air (0-1%), dan laju aliran (100-300 SSL / minit) telah diselidiki untuk pengoptimuman dari respon luas puncak LC dan isyarat terhadap noise (S/N). Tanggapan dioptimumkan diperolehi dengan menggunakan syarat berikut: pelarut organik 5 di awal dan 95% pada akhir fasa gerak kecerunan, konsentrasi asid 0,1%, dan 250 SSL / min laju aliran. Selain itu, prosedur pembuatan sampel terbaik telah dipilih oleh menilai kesan daripada dua jenis umum yang berbeza kaedah ekstraksi pelarut (satu langkah dan dua langkah ekstraksi) dan empat jenis pembersihan kaedah termasuk Oasis HLB, MycoSep, medan immunoaffinity (IAC) dan tidak ada bersih-up pada pemulihan mikotoksin. Keputusan kajian menunjukkan bahawa pemulihan terbaik (79-109%) untuk semua mikotoksin akan diperolehi dengan menggunakan salah satu langkah ekstraksi tanpa membersihkan. Akhirnya, kaedah LC-MS/MS dioptimumkan disahkan dengan mengukur selektivitas, sensitiviti, Linieritas, ketepatan dan presisi. Tarikh pengesanan (LOD) untuk AFB_1, AFB_2, AFG_1, AFG_2, DON, T2-Toxin, HT2-Toksin, FB_1, FB_2, OTA dan Zea adalah 0.05, 0.25, 0.05, 0,5, 5, 2, 2, 10, 10 , 0,01, dan 0,1, sedangkan Batas kuantifikasi (loq) adalah 0,1, 0,5, 0,1, 1, 10, 4, 4, 20, 20, 0.02, dan 0.2 ppb, masing-masing. Akhirnya, dioptimumkan dan diaktifkan LC-MS/MS kaedah ini diterapkan pada contoh nyata bijirin (beras, barley, oat, gandum dan jagung) yang dikumpulkan dari pasaran Malaysia. Keputusan kajian

menunjukkan penerapan kaedah untuk digunakan sebagai kaedah rutin dengan ketepatan dan presisi tinggi untuk penentuan serentak dari mikotoksin pada bijirin.

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I certify that a thesis Examination Committee met on 22 April 2011 to conduct the final examination of Farhang Soleimany on his thesis entitled "Development, Optimization and Validation of LC-MS/MS Method for Multi-mycotoxin Detection in Cereals" in accordance with the Universities and University College Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U. (A) 106] 15 March 1998. The committee recommends that the student de awarded the Doctor of Philosophy.

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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 Doctor of Philosophy. 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 UPM or at any other institutions.

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FARHANG SOLEIMANY

Date: 22 April 2011

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