DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC METHODS FOR THE DETERMINATION OF MYCOTOXINS IN FOOD

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Specially dedicated to:

My mother, wife, daughters and other family members for all their love, support and continuous prayer for my success in completing this work.

ABSTRACT

The development and validation of analytical techniques to be applied for the determination of naturally occurring mycotoxins has been the focus of this study. The structure of this work is divided into four main parts, from which independent conclusions are drawn. The first part is in the development of a rapid, reliable and confirmatory method to determine the levels of aflatoxins B1, B2, G1, and G2 in barley, wheat, soybeans and corn. This method is based on a single extraction step followed by liquid chromatography coupled with electrospray ionization quadrupole time of flight mass spectrometry (LC-ESI-QTOF-MS/MS). The sensitivity of the ESI interface was significantly enhanced by optimizing the chromatographic conditions and the fragmentor voltage in the interface. By using the mycotoxin database table, the mycotoxins were confirmed by their retention times, the accurate mass measurements of the TOF analyzer and the products ions, thus avoiding false-positive results. The quantification of the analytes was carried out by performing low-energy collision induced tandem mass spectrometry (CID-MS/MS) using the multiple reaction monitoring (MRM) mode. Secondly, the implementation and validation of the optimized LC-ESI-QTOF-MS/MS method and the development of a new method based on Quick, Easy, Cheap, Effective, Rugged, and Safe (QuEChERS) technique for the determination of eight(8) type-A and type-B trichothecenes in cereal samples are carried out. The recovery results showed that the developed QuEChERS method was effective in removing unwanted interfering components without any further clean-up procedure. Therefore, the use of dispersive

solid-phase extraction (dSPE) cleanup step was excluded to reduce the time and cost of analysis. The third part is focused on optimizing the sample pretreatment conditions of the developed method and the optimization of the chromatographic conditions of the HPLC-FLD method with postcolumn photochemical derivatization for the quantification of four(4) aflatoxins B1, B2, G1 and G2 in food. The extraction solvent was found to be the most important factor as it significantly affects the extraction efficiency. On the other hand, it was found that the wavelengths setting at 365nm excitation and 440nm emission could be used as the optimum wavelengths for all aflatoxins. The developed QuEChERS-HPLC method was then validated and compared with the standard fluorometric determination method. It was found that the fluorometric determination method showed a poorer precision and a positive bias when compared to the QuEChERS-HPLC method. The QuEChERS-HPLC method was then used for the analysis of the selected aflatoxins in a total of 669 domestic and imported food samples in Jordan. Peanut and peanut butter samples showed the highest incidence of contamination (10 contaminations) followed by pistachio nut samples (6 contaminations) and sesame seed samples (2 contaminations). The final component of this study is the implementation and validation of the optimized QuEChERS-HPLC method for the determination of ochratoxin A in cereal samples. Excellent linearity, high recoveries and acceptable precision with the LOQ values, which are lower than the stipulated Maximum Residue Level (MRL), were achieved indicating the suitability of the proposed methods for the determination of mycotoxins in foods could be implemented for routine analysis.

ABSTRAK

Perkembangan dan pengesahan teknik analisis yang digunakan untuk penentuan mikotoksin semulajadi telah menjadi fokus kajian ini. Struktur kerja ini dibahagikan kepada empat bahagian utama, dari mana kesimpulan yang berdikari telah disediakan. Bahagian pertama melibatkan perkembangan satu kaedah yang pantas, diandalkan dan pengesahan untuk menentukan paras aflatoksin B1, B2, G1, G2 dalam barli, gandum, kacang soya dan jagung.Kaedah ini adalah berasaskan kepada langkah pengekstrakan tunggal diikuti oleh kromatografi cecair yang berserta dengan masa kuadrupol pengionan electrospray penerbangan spektrometri jisim (LC-ESI-QTOF-MS/MS).

Kepekaan antara muka ESI telah meningkat dengan ketara melalui yang mengoptimumkan syarat kromatografi dan voltan fragmentor yang dalam antara muka. Dengan menggunakan jadual pangkalan data mikotoksin, mikotoksin boleh disahkan oleh masa tahanan mereka, ukuran jisim yang tepat dari penganalisa TOF dan ion produk, sekaligus mengelakkan keputusan palsu-positif. Kuantifikasi daripada analit diukur dengan melakukan perlanggaran rendah tenaga teraruh sejajar spektrometri jisim (CID-MS/MS) menggunakan pemantauan tindak balas pelbagai mod (MRM). Kedua, pelaksanaan dan pengesahan kaedah LC-ESI-QTOF-MS/MS dioptimumkan dan perkembangan kaedah baru yang berdasarkan Teknik Pantas, Mudah, Murah, Berkesan, Lasak, Dan Selamat (QuEChERS) untuk menentukan lapan(8) jenis A dan jenis B trichothecenes dalam sampel bijirin dijalankan. Keputusan pemulihan menunjukkan bahawa kaedah QuEChERS adalah berkesan dalam menghapuskan komponen gangguan

tanpa prosedur lanjut pembersihan. Oleh itu, penggunaan pengekstrakan serakan fasa pepejal (dSPE) bagi langkah pembersihan tidak termasuk untuk mengurangkan masa dan kos analisis. Bahagian ketiga tertumpu kepada mengoptimakan syarat-syarat prarawatan sampel yang ditentukan dan pengoptimuman syarat-syarat kromatografi dari kaedah HPLC-FLD dengan derivatisasi postkolum foto kimia untuk menghitung sebanyak empat (4) aflatoksin B1, B2, G1 dan G2 di dalam makanan. Pemisahan pelarut didapati menjadi faktor yang paling penting kerana ketara memberi kesan kepada kecekapan pengekstrakan. Sebaliknya, ia telah mendapati bahawa panjang gelombang yang menetapkan pada pengujaan 365nm dan pemancaran 440nm boleh digunakan sebagai panjang gelombang optimum untuk semua aflatoksin. Kaedah QuEChERS-HPLC yang dikembangkan kemudian disahkan dan berbanding dengan kaedah penentuan fluorometrik yang biasa. Ia telah mendapati bahawa kaedah penentuan fluorometrik mempunyai ketepatan yang kurang dan berat sebelah positif berbanding kepada kaedah QuEChERS HPLC. Kaedah QuEChERS- HPLC kemudiannya digunakan untuk analisis aflatoksin terpilih dalam sejumlah 669 sampel makanan domestik dan import di Jordan. Sampel mentega kacang dan kacang menunjukkan insiden kontaminasi tertinggi (10 kontaminasi) diikuti oleh sampel kacang pistachio (6 kontaminasi) dan sampel biji bijan (2 kontaminasi). Komponen kajian terakhir ini adalah pelaksanaan dan pengesahan kaedah QuEChERS-HPLC dioptimumkan bagi penentuan ochratoxin A dalam sampel bijirin. Kelinearan yang cemerlang, kutipan semula yang tinggi dan ketepatan yang boleh diterima dengan nilai LOQ, yang adalah lebih rendah daripada yang ditetapkan untuk Residu Tahap Maksimum (MRL) telah dicapai menunjukkan kesesuaian kaedah yang dicadangkan untuk menentukan mikrotoksin dalam makanan yang boleh dilaksanakan untuk analisis rutin.

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LIST OF ABBREVIATIONS

15-ADON	15-Acetyldeoxynivalenol
3-ADON	3-Acetyldeoxynivalenol
ADON	Acetyldeoxynivalenol
ANOVA	Analysis of Variance
AOAC	Journal of Association of Official Analytical Chemists
C18 silica	Octadecylsilane
CE	Capillary Electrophoresis
CEN	European Committee of Standardization
DAD	Diode Array Detector
DAS	Diacetoxyscirpenol
DON	Deoxynivalenol
dSPE	Dispersive Solid Phase Extraction
ELISA	Enzyme-Linked Immunosorbent Assay
EDI	Electrodeionization
ESI	Electrospray Ionization Source
EU	European Union
FAO	Food and Agriculture Organization
FLD	Fluorescence Detector
FUS X	Fusarenon-X
GC	Gas Chromatography
GC x GC-TOF-MS	Two-Dimensional Gas Chromatography-Time-Of-Flight Mass Spectrometry
GC-MS	Gas Chromatography Coupled with Mass Spectrometry
HETP	Theoretical Plate Number / Column Length
HFB	Heptafluorobutyl
HFBA	Heptafluorobutyric Anhydride
HPLC	High Performance Liquid Chromatography
HPLC-DAD	High Performance Liquid Chromatography Coupled with Diode Array Detector
HPLC-FLD	High Performance Liquid Chromatography Coupled with Fluorescence Detector
HT-2	HT-2 Toxin
IAC	Immunoaffinity Column
IARC	International Agency for Research on Cancer
JECFA	Joint FAO / WHO Expert Committee on Food Additives
JISM	Jordan Institution for Standards & Metrology
LC-ESI-MS	Liquid Chromatography coupled with Electrospray Ionization-Mass Spectrometry
LC-ESI-QTOF-MS/MS	Liquid Chromatography coupled with Electrospray Ionization Quadrupole Time Of Flight Mass Spectrometry
LC-MSMS	Liquid Chromatography Coupled with Tandem Mass Spectrometry

LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LSE	Liquid-Solid Extraction
NIV	Nivalenol
NPLC	Normal-Phase Liquid Chromatography
PFP	Pentfluoropropyl
PFPA	Pentafluoropropionic Anhydride
Rf	Retention Factor
PHRED	Photochemical Reactor for Enhanced Detection
PSA	Primary and Secondary Amine
RPLC	Reversed-Phase Liquid Chromatography
RSD	Relative Standard Deviation
S/N	Signal-to-Noise Ratio
SFE	Supercritical Fluid Extraction
SIM	Selected Ion Monitoring
SPE	Solid-Phase Extraction
T-2	T-2 Toxin
TFA	Trifluoroacyl
TFAA	Trifluoroacetic Acid Anhydride
TLC	Thin Layer Chromatography
TMS	Trimethylsilyl
UVD	Ultraviolet-Visible Detection
UV-Vis	Ultraviolet-Visible