شبكة المعلومات الجامعية

بسم الله الرحمن الرحيم

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

Study for quality control of meat products, meat speciation (halal test, meat adulteration) and veterinary drug residues using LC-MS/MS

Thesis Submitted

By

Mahmoud Abd elkhabier Mohamed
M.Sc. in Chemistry
Faculty of Science, Ain Shams University
2016

In the Partial Fulfillment for the Requirement of the PhD Degree in Chemistry

Chemistry Department, Faculty of Science
Ain Shams University

Under Supervision of

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Approval sheet

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Abstract

Name: Mahmoud Abd elkhabier Mohamed

Title of the thesis: Study for quality control of meat products, meat speciation (halal test, meat adulteration) and veterinary drug residues using LC-MS/MS

Position: Laboratory chemist

Degree: PhD, Faculty of science, Ain Shams University

A sensitive, robust, and selective analytical method for determination of 17 Sulfonamides (SAs), 4 Tetracyclines (TCs) and Chloramphenicol (CAP) residues to be used in monitoring program for the analysis of studied veterinary drugs in chicken samples from different poultry farms of Egypt by cutting-edge LC-MS/MS QTRAP has been established and validated. Sample treatment based on QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) with several adjustments depending on the matrix and drugs group. Double extractions using acetified acetonitrile with 1% acetic acid are common practice in sample preparation of sulfonamides (SAs) and chloramphenicol (CAP) while extraction for tetracyclines (TCs) was by 70% methanol/DIW and pH adjusted by formic acid to be four. Extracts were cleaned-up using PSA for the dispersive solid

phase extraction step for interference removal. Various conditions such as MS compatible solutions, LC column, mobile phase and gradient elution tested in order to save analysis time and for efficient chromatographic separation. Optimization of the various MS/MS experimental parameters (Declustering potential, collision cell exit potential, desolvation temperature, gas flow and collision energy) also carried out by full scan and selective reaction monitoring (SRM) with direct injection of single and mixed standard solutions. Matrix interferences monitored in extracts of honey, chicken and pork muscles for 22 antibiotics using recently developed and validated methods. Although a significant dispersion in the results observed for most of the compounds, ion suppression was the major issue. The optimized method validated, obtaining suitable results for all validation parameters in the evaluated matrices. Instrument linearity was established using multi-level calibration curve from 1-100µg/L for sulfonamides and tetracyclines and from 0.1-20µg/L for chloramphenicol; the correlation coefficient was $\geq$ 0.995 for all compounds. Methods linearity studied using different concentration levels, which lie in between the calibration points. The method proved to be linear for all compounds from Limit of quantitation (LOQ) to the highest level. Limit of quantitation was 10µg/L for Sulfonamides,

Tetracyclines, and 0.2µg/L for chloramphenicol. Recovery values ranged from 70%-120%. Repeatability and reproducibility obtained at values lower than 20%. The limit of quantification (LOQ) established at 0.20, 10 and 15 µg/kg for CAP, SAs and TCs, respectively, which were lower than the maximum residue limit legally established by the European Union (EU). Method accuracy studied using various certified reference materials (CRMs) and proficiency tests (PTs) samples, results found to be valid within the acceptable limits. A full in-house validation of the method intended for routine analysis to support regulatory enforcement. Sixty fresh samples from different poultry farms in Egypt tested for the presence of studied compounds. Results showed that there were no positive samples detected except two samples contaminated with doxycycline but lower than the maximum residue limits (MRLs) established by European database (100µg/kg). Hence, more monitoring and risk assessment studies required to cover new generations of veterinary drugs.

Key words
Food of animal origin, LC-MS/MS, method optimization, QuEChERS, Veterinary drugs, Meat adulteration.

Supervisors' approval

- **Prof. Dr. Eglal Maryiam Raymon Souaya**

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- **Dr. Lamia Ryad**

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Signature: ……

Head of Chemistry Department
Prof Dr. Ayman Ayoub Abdel-Shafi

Aim of study

Due to the importance of controlling the quality of meat in Egypt and estimating the risks that may occur due to the residues of veterinary drugs, this study conducted to achieve the following objectives:

- To optimize and validate different methods for determination of 17 sulfonamides, SAs (sulfacetamide, sulfadiazine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfapyridine, sulfathiazole, sulfachloropyradazine, sulfadimethoxine, sulfadoxine, sulfisoxazole, sulfaquinoxaline, sulfamethizole, sulfamoxole, sulfaguanidine, sulfamonomethoxine, sulfamethoxypyradazine,) and 4 tetracyclines, TCs (chlortetracycline, oxytetracyclin, doxycyclin and tetracycline) as well as chloramphenicol (CAP) in chicken meat using modified QuEChERS and LC-MS/MS QTRAP.
- Matrix Effect Study of Chicken Co-extractives in the studied antimicrobial drugs using Ultrahigh-Performance LC-MS/MS.
- Detecting and estimating the level of antimicrobial drug residues in chicken samples collected from different farms in Egypt.
- Conduct risk assessment studies based on samples collected and analyzed from different farms in Egypt.
- Develop a new method for detection of meat adulteration using five meats: beef, pork, chicken, duck and lamb, using biomarker peptides by mass spectrometry-based protocol at levels of 0.1% (w/w) with high sensitivity and specificity.

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