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固体表面荧光－CCD 数码成像法在快速检测鱼肉中四环素类抗生素残留中的应用研究

Application of CCD Camera Imaging Based Solid Surface Fluorescence for Fast Screening of Tetracyclines in Fish Muscle

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摘 要

水产品中渔药残留检测具有重要意义，其不仅关系到消费者的身体健康，而且也是应对发达国家的贸易绿色壁垒，发展我国海洋经济的关键技术之一。目前，在水产品渔药残留检测中存在的最大问题是分析耗时较长，成本和技术要求高，不适合大批量样品的现场快速检测。基于此，本论文工作以四环素类抗生素为研究对象，开发快速筛选水产品中四环素类抗生素残留总量是否超标的分析方法。论文共分为四章，包括前言、固体表面荧光－CCD 成像法测定四环素类抗生素、固体表面荧光－CCD 成像法在测定水产品中四环素类抗生素残留的应用及结论与展望。

第一章，前言，通过文献调研，对水产品渔药残留分析的意义及研究现状进行了归纳和总结；综述了近年来水产品中四环素类抗生素残留的分析方法；介绍了固体表面荧光分析法的原理及应用现状，并在此基础上提出了论文的研究思路。

第二章，固体表面荧光－CCD 成像法测定四环素类抗生素。四环素类抗生素属弱荧光物质，不能在荧光仪上直接检测，一般需要经过荧光衍生化反应，本课题组基于四环素类抗生素固相碱性降解发光，结合自行设计的 CCD 数码成像检测系统，建立了四环素类抗生素的固体表面荧光－CCD 成像检测法。本论文在课题组前期工作的基础上对实验条件及测定方法进行了进一步优化，提出了复式点样技术，该技术通过多次重复点样对四环素类抗生素进行有效的富集，从而提高了方法的灵敏度，有利于实际样品中低浓度残留量的检测。方法具有简单、快速、取样量少、灵敏度高、费用少等特点。实验结果表明，四环素类抗生素的线性范围均为 0.20～1.0 ng $spot^{-1}$，根据三倍信噪比与十倍信噪比，四环素、土霉素、金霉素的检测限分别为 0.14，0.15，0.16 ng $spot^{-1}$，定量限分别为 0.47，0.50 and 0.53 ng $spot^{-1}$

第三章，固体表面荧光－CCD 数码成像法在快速检测水产品中四环素类抗生素残留的应用。空白鱼样加标实验结果表明该方法的线性范围为 50～300 μg

Kg^{-1}，相关系数 R = 0.997；加标量为 50，100，200 μg Kg^{-1}（分别为最大残留限量的 0.5，1，2 倍）6 种不同鱼类样品（罗非鱼、鲈鱼、白鲫鱼、鲳鱼、黄花鱼、金线鱼）的平均回收率为 63.1%，变异系数为 8.2%；方法的日内重现性 RSD = 2.5～5.8% , 日间重现性 RSD = 4.9～7.6%；方法的检测限与定量限分别为 16、53 μg Kg^{-1}；根据欧盟 2002/657/EC 决议，判定限（CCα）和检定能力（CCβ）分别为 107、114 μg Kg^{-1}；根据均值－标准差质量控制图，该方法可用作判断水产品中四环素类抗生素残留总量是否超标的快速筛选手段，；与高效液相色谱法相比，本方法具有简单、快速、费用低等特点，适合于大量样品的快速现场检测。

第四章，对研究工作做出总结，指出其不足之处，并对该课题的研究前景进行了展望。

关键词：四环素；固体表面荧光法；CCD 成像检测；渔药残留

Abstract

Tetracyclines, (TCs) are broad-spectrum antibiotics showing activity against Gram-positive and Gram-negative bacteria including some anaerobes, by inhibiting the synthesis of bacterial proteins by binding to the 30 S bacterial ribosome and preventing the access of aminoacyl tRNA to the acceptor site on the mRNA–ribosome complex. TCs have been widely used in the prevention and treatment of infectious diseases, and as food additives for growth promotion as well, in the fish farming. The intensively use of these antibiotics have raised questions about the impact of veterinary medicines on organisms in the environment and on human health. Numerous studies suggest a link between antibacterial use in agriculture and antibacterial-resistant infections, and there is evidence that antibacterial resistance from agriculture can be transferred to humans. It is important to develop sensitive analytical methods to monitor the food supply to ensure that any antibiotic residues present are below the set tolerance level, thus promoting food safety and consumer confidence. A maximum residual limit of 100 μg Kg^{-1} of TCs in aquiculture product is set by EU and China.

This thesis is aimed at developing a new methodology for fast screening of TCs in fish muscle. Four chapters are included in this thesis, that is, introduction, method development and optimization, screening of TCs in fish muscles, and conclusion and expectation.

In Chapter 1, a brief introduction of the use and effect of fishery drug in aquiculture and a review discussing the present stage of the art of the quantificational and qualitative methodology for TCs residues in aquatic product are given. A basic principle of solid-surface fluorescence (SSF) and its applications are introduced. Based on the update development in these areas, the research plan for the thesis was outlined.

In Chapter 2, a previous developed quantificational method for the determination of TCs was optimized. This methodology is based on the fact that tetracyclines would transfer to highly fluorescent species on alkaline active solid silica gel G plates as a result of their alkaline degradation. By coupling the solid surface fluorescence with a CCD camera imaging, a CCD camera based SSF has been developed. In order to optimize the performance, the experiment parameters that would effect the result of determination were optimized. Being implemented with a multiple-spotting technique the sensitivity of this method was increased obviously, and the laborious time was also shorted. The method has demonstrated to bear some requiring advantages, such as its simplicity, rapidness, low-cost, less pollutants and reasonable sensitivity, on comparison with the official methods, HPLC. The calibration curves give linearity over a range of 0.20~1.0 ng $spot^{-1}$ for all three most used tetracyclines, named tetracycline (TC), oxytetracycline (OTC) and chlorotetrecycline (CTC). The limits of detection (LOD), defined as 3σ, for TC, OTC and CTC are 0.14, 0.15 and 0.16 ng $spot^{-1}$, respectively. The limit of quantification (LOQ), defined as 10σ, for TC, OTC and CTC are 0.47, 0.50 and 0.53 ng $spot^{-1}$, respectively.

In Chapter 3, the newly developed CCD camera based SSF was applied to the screening of the tetracyclines residues in fish muscle samples. The method was validated by evaluating the performance criteria: recovery, repeatability, calibration curve, limit of detection (LOD) and limit of quantification (LOQ), decision limit (CCα) and detection capability (CCβ), false positive and false negative rate, sensitivity and specificity rate, in accordance with European Commission Decision 657/2002. The average percentage recovery for fish muscle sample from 6 different fish matrix, named *tilapia, weeever, crucian, silver carp, silver pomfret and besugo*, fortified with TC at different level, was 63.1% with an average coefficient of variation of 8.2%. The coefficients of variation ranged from 2.5 to 5.8% and from 4.9 to 7.6% for intra-day and inter-day repeatability, respectively. The detection and quantification limit in fish muscle matrix was 16 and 53 μg Kg^{-1} of TCs, respectively. CCα and CCβ

were found to be 107 and 114 μg Kg^{-1}, respectively, determined on a basis of a MRL of 100 μg Kg^{-1}. The calibration curve in different fish muscle matrix showed a good linearity in the whole range of tested concentrations (50~300 μg Kg^{-1}) with a correlation coefficient (R) equal to 0.997. Setting a threshold as the $\bar{x}_{100}$ - $3\sigma_{100}$ allows for successful screening of TC in fish muscle fortified at 100 μg Kg^{-1}, maximum tolerance level set by Ministry of Agriculture of the People's Republic of China. For 50 blank fish samples, each fortified at a randomly selected level in the range of 0～200 μg Kg^{-1}, the sensitivity rate, specificity rate, false positive rate and false negative rate was 100%, 88.9%, 11.1% and 0%, respectively. This method can provide an easy, fast, and low-cost screening assay compared with HPLC.

In Chapter 4, the research work was summarized and the prospect of the research field was given.

Key Word: Tetracycline; Solid Surface fluorescence; CCD Imaging Analysis; Fishery Drug Residues

第一章 概述

1.1 水产品渔药残留分析

1.1.1 渔药及渔药残留简介[1]

渔药是用来预防与治疗水产养殖对象疾病，协助其机体恢复正常功能或促进其机体健康成长的药物。根据其作用对象，可分为水产植物药和水产动物药；根据其用途，可分为抗微生物药（抗生素类、磺胺类、呋喃类）、消毒杀菌药、环境改良药、抗寄生虫药、营养保健药、激素以及生物制品；根据其化学组成，又可分为无机药、有机药以及生物类药。

渔药残留是指在水生动、植物养殖过程中，所使用的渔药因积累或代谢不完全在生物体内留存的原型化合物以及其代谢产物，包括与药物本体有关的杂质。目前国际上比较重视的渔药残留有抗生素类、磺胺类、呋喃类、喹诺酮类、激素类和转寄因类药物等。

1.1.2 水产品渔药残留的影响

1.1.2.1 对人体健康的影响[1-3]

水产业的发展，离不开渔药的使用。药物添加剂的长期使用，导致其在动物体内积蓄。水产品中渔药残留引发急性中毒的可能性较低，但长期低剂量的摄入，可导致各种慢性和积蓄性疾病，还会产生耐药性，对人体健康的潜在危害甚为严重，而且影响深远。近年来有关药物残留的危害的报道越来越多，现就以下几个主要方面加以介绍。

1.1.2.1.1 毒性作用

当水产品中药残在人体内蓄积到一定浓度时会对人体产生毒性作用。例如人体长期接触氯霉素而造成富集，会影响其造血功能，导致粒细胞缺乏症，引起再生性障碍性贫血即白血病；磺胺类药物及其代谢的中间物特别是乙酰化磺胺可对

肾脏造成严重损害；用于杀死鱼体外的鞭毛虫、纤虫、吸管虫常用的是硫酸铜、敌百虫，硫酸铜过量可造成鱼体内金属积累，敌百虫在弱碱下可形成具有强毒性的敌敌畏。

1.1.2.1.2 过敏反应和变态反应

一些残留药物如青霉素、磺胺类、四环素类等具有抗原性、能刺激机体形成抗体产生过敏反应和变态反应。人们长期食用含有磺胺类、四环素类的水产品后，会出现多种过敏反应及变态反应，轻者表现为皮炎、喉咙水肿、发热，重者会出现白细胞减少、溶血性贫血，甚至发生危及生命的综合症。

1.1.2.1.3 造成体内细菌耐药性增加

水产品反复接触某些药物后，其体内的敏感菌株会受到选择性抑制，细菌产生耐药性，使耐药菌株大量繁殖，当人食用含有药物残留的水产品，水产品内的耐药菌株可传递给人体。目前，人们已经发现了不少可以同时抵御多种抗生素（如氯霉素、四环素、磺胺类链霉素等）的沙门氏菌株、金黄色葡萄球菌耐药菌株等。耐药菌株带给人体的危害是深远的，耐药菌株在人体内的繁殖给人体疾病临床治疗带来困难。

1.1.2.1.4 三致作用

部分水产品的药残有致畸、致突变、致癌作用。例如用于防治细菌疾病和虾、蟹类寄生虫的消毒杀菌剂中含有氯，氯可与水中有机物质反应生成致癌物质；孔雀石绿可防治鱼卵的水霉病、车轮虫病等，但孔雀石绿具有强毒性，其残留有致畸、致癌作用；己烯雌酚有诱发女性乳腺癌、卵巢癌等危害。长期食用含有三致作用药残水产品便会对人体产生有害作用，最终导致三致。

1.1.2.1.5 性早熟作用

激素类用于育苗期性转变，常用的有己烯雌酚、去甲睾丸酮等。激素类渔药残留会使正常人的生理功能发生紊乱，干扰人体激素系统正常运行，并发肥胖，危