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硕 士 学 位 论 文

环境水样中 24 种抗生素残留的同时分析方
法及其应用研究

Study on Determination Method for 24 Antibiotics in
Aquatic Environment Samples and Its Application

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厦门大学博硕士论文摘要库

摘要

随着畜禽、水产养殖业的快速发展, 抗生素的种类和用量日益增加。抗生素在动物性产品中的残留不仅引起了食品安全问题, 相当一部分还随饲料投放、动物粪便、医药废物以及降水冲刷等过程进入水环境中, 给环境安全和生态健康施加了风险。本论文针对养殖业常用的 24 种抗生素, 采用固相萃取-超高压液相色谱-串联质谱检测方法建立了其在河水和海水中的高灵敏、同时分析方法, 并成功运用于九龙江口及厦门近岸海域表层水中抗生素残留状况的调查。

主要研究内容和结果如下:

(1) 24 种抗生素超高压液相色谱-串联质谱检测方法的建立

运用超高压液相色谱串联质谱仪, 在多反应选择监测 (MRM) 模式下, 通过优化毛细管电压、锥孔电压、碰撞能等质谱参数和流动相组成、梯度洗脱程序等色谱分离条件, 建立了 24 种抗生素的高灵敏、定性定量准确而又快速有效的仪器分析方法。除四环素类抗生素为 $10 \mu\text{g/L}$ 外, 其余目标物的仪器测定限均小于 $0.5 \mu\text{g/L}$; 各类抗生素的线性范围分别为氯霉素类 $0.5 \sim 1000.0 \mu\text{g/L}$ 、四环素类 $10.0 \sim 1000.0 \mu\text{g/L}$ 、喹诺酮类和磺胺类 $0.2 \sim 1000.0 \mu\text{g/L}$; 线性相关系数为 $0.9982 \sim 0.9999$ 。本检测方法具有定性准确、灵敏度高、重现性好、简单快速的优点 (24 种抗生素分三组检测, 整个仪器分析过程可在 1 h 内完成)。

(2) 河水和海水中 24 种抗生素固相萃取前处理方法的建立

以河水和海水为基底, 利用 HLB 固相萃取柱建立了步骤简单、富集/净化效果较好的 24 种抗生素残留的预处理方法。实验考察了 5 种不同固相萃取填料对水中目标抗生素的富集能力, 最后选择 HLB 固相萃取柱并优化了水样 pH、盐度、过柱体积、过柱速度、洗脱溶剂和洗脱体积等固相萃取条件。在最佳实验条件下, 5.0 、 20.0 和 100.0 ng/L 的低加标浓度时, 本方法中各目标抗生素以河水为基底的加标回收率为 $50.1\% \sim 94.7\%$, 相对标准偏差 (RSD, $n=4$) 为 $1.6\% \sim 9.7\%$; 以海水为基底的加标回收率为 $46.7\% \sim 81.2\%$, 相对标准偏差 (RSD, $n=4$) 为 $4.8\% \sim 9.4\%$ 。除四环素类抗生素的方法

检测限为 20.0 ng/L 外,其余抗生素的方法检测限均可达到 1.0 ng/L 甚至更低。结合替代物质控方法的使用,方法可以满足水中痕量 (ppt 级) 抗生素残留的检测要求。

(3) 九龙江口及厦门近岸海域表层水中 24 种抗生素的残留状况研究

分别于 2006 年 12 月和 2007 年 4 月两次采集九龙江口及厦门近岸海域 28 个站位的表层水样,运用建立的固相萃取-超高压液相色谱串联质谱 (SPE-UPLC-MS-MS) 法进行了 24 种抗生素残留的分析。结果显示,氟甲砜霉素 (2.1~23.0 ng/L)、磺胺嘧啶 (1.8~162.7 ng/L)、磺胺二甲基嘧啶 (1.7~153.3 ng/L)、磺胺间甲氧嘧啶 (1.5~50.2 ng/L) 和磺胺甲恶唑 (4.2~40.8 ng/L) 在调查区域的全部 28 个站位中均被检出;氯霉素 (0.5~5.9 ng/L) 和磺胺对甲氧嘧啶 (1.2~11.3 ng/L) 在大部分站位被检出;甲砜霉素 (1.0~2.6 ng/L) 和磺胺吡啶 (0.3~2.0 ng/L) 只在九龙江口附近的站位中被检出;氧氟沙星 (0.9~5.8 ng/L) 只在厦门近岸海域的站位中被检出。与国外一些研究只在河流的局部区域检出少数抗生素的结果相比,九龙江口流域和厦门近岸海域的抗生素污染值得关注。

关键词: 抗生素; 固相萃取; 超高压液相色谱串联质谱 (UPLC-MS-MS); 九龙江流域; 厦门近岸海域; 河水; 海水。

ABSTRACT

With the rapid development of livestock agriculture and aquaculture, the types and dosage of antibiotics is keeping on increasing. The antibiotic residues in animal products have not only caused food safety problems, but also imposed pressures on environmental health and ecosystem security, as considerable amount of them being released into water environment through the disposal of animal feces, medical wastes and direct feeding jettison. In this work, a method for simultaneous determination of 24 commonly used antibiotics in river and sea water samples using solid phase extraction and ultra performance liquid chromatography coupled with electrospray tandem mass spectrometry (SPE-UPLC-MS-MS) has been developed and successfully applied to survey the distribution of target antibiotic residues in Jiulong River estuary and Xiamen coastal area. The research contents and results are as the followings:

(1) Establishment of Simutaneous detection method for 24 antibiotics using ultra performance liquid chromatography coupled with electrospray tandem mass spectrometry

A high sensitive, accurate and effective method for the simultaneous detection of 24 antibiotics using ultra performance liquid chromatography with electrospray tandem mass spectrometry (UPLC-MS-MS) under multiple reaction monitoring mode (MRM) was developed. The effects of composition and gradient elution program of the mobile phase on the separation and MS parameters, such as capillary voltage, cone voltage and collision energy were investigated and optimized. Under the optimized conditions, the detection limits of antibiotics all reached 0.5 $\mu\text{g/L}$, except tetracyclines at 10 $\mu\text{g/L}$. The linearity ranges of chloramphenicols, tetracyclines, quinolones and sulfonamides were 0.5 to 1000.0 $\mu\text{g/L}$, 10 to 1000.0 $\mu\text{g/L}$ and 0.2 to 1000.0 $\mu\text{g/L}$, respectively. The correlation coefficients were all between 0.9982 and 0.9999. The method is high sensitive, reproducible, simple and fast (24 target antibiotics were divided into three groups

and the entire detection process could be accomplished within 1 h).

(2) Development of solid phase extraction method for simultaneous extraction of 24 antibiotics from river and sea water samples

A simple and enrichment/cleanup-effective solid phase extraction method for the simultaneous extraction of 24 antibiotics in river and sea water samples using HLB cartridge was developed. The enrichment and cleanup effects of 5 different types of SPE cartridges were compared, and various influential factors such as pH and salinity of water samples, breakthrough volume, flow rate and the elution solvent and volume, were investigated. Under the optimized conditions, recoveries and relative standard deviations (RSD, n = 4) at 5.0 ng/L, 20.0 ng/L and 100.0 ng/L spiking levels were 50.1%~94.7%, 1.6%~9.7% for river water matrix, 46.7%~81.2%, 4.8%~9.4% for seawater matrix, respectively. The method detection limits of target antibiotics could reach 1.0 ng/L, even lower with the exception of tetracyclines at 20 ng/L. Using surrogates as quality control method, the proposed method could meet the requirement for detecting trace antibiotic residues in aquatic environments.

(3) Distribution survey of 24 antibiotics in Jiulong River estuary and Xiamen coastal area surface water

The existence and distribution of 24 antibiotics in Jiulong River estuary and Xiamen coastal area surface water were surveyed in December 2006 and April 2007 using established SPE-UPLC-MS-MS method. Results indicated that florfenicol (2.1~23.0 ng/L), sulfadiazine (1.8~162.7 ng/L), sulfamethazine (1.7~153.3 ng/L), sulfamonomethoxine (1.5~50.2 ng/L) and sulfamethoxazole (4.2~40.8 ng/L) were detected in all of the 28 sampling stations, chloramphenicol (0.5~5.9 ng/L) and sulfameter (1.2~11.3 ng/L) were detected in most sampling stations, thiamphenicol (1.0~2.6 ng/L) and sulfapyridine (0.3~2.0 ng/L) were found only in sampling stations near Jiulong River estuary, and ofloxacin (0.9~5.8 ng/L) was found only in sampling stations near Xiamen coastal area.

Compared with the research results from foreign countries, the status of antibiotic pollution in Jiulong River estuary and Xiamen coastal area should be of great concern.

Keywords: antibiotic; solid phase extraction (SPE); ultra performance liquid chromatography coupled with tandem mass spectrometry (UPLC-MS-MS); Jiulong River estuary, Xiamen coastal area; river water; sea water.

厦门大学博硕士学位论文摘要库

第1章 绪论

与化学肥料、农药、多氯联苯、POPs 等化学品所引起的环境问题受到的长期、广泛关注相比，抗生素作为人们日常生活接触最为频繁、用量最大的一类化学品，其环境行为和效应直到 1990 年代后期才受到关注^[1]。这类药物通常具有与许多有害的外源性物质（Xenobiotics）相似的结构和理化行为，如能够穿过细胞膜的亲脂性基团；较高的稳定性以避免在产生治疗效应前失活；易于产生生物累积等。其通过多种途径进入环境后，可能抑制环境中有益微生物的活性、刺激病原菌产生抗药性，从而对陆生或水生生态系统产生负面效应^[1-2]。

1.1 主要抗生素类药物及其使用状况

1.1.1 主要的抗生素类药物

抗生素（Antibiotics）是生物，包括微生物、植物和动物在内，在其生命活动过程中所产生的（或由其他方法获得的），能在低微浓度下有选择地抑制或影响它种生物功能的有机物质^[3-4]。自 1940 年青霉素应用于临床以来，人类开始广泛使用抗生素，目前临床上常用的即达数百种^[5]。抗生素在细菌性疾病的治疗中发挥了极其重要的作用，目前主要类型如下：

（1）氯霉素类抗生素：一种由委内瑞拉链霉菌中分离提取的广谱抗生素。对许多需氧革兰氏阳性细菌和革兰氏阴性细菌、厌氧的拟杆菌、立克次氏体、衣原体及菌质体都有抑制作用，尤其对沙门氏菌属、流感杆菌和拟杆菌属等有良好的抗菌能力。氯霉素使用不当可致新生儿血液循环障碍、呼吸功能不全、发绀、腹胀（即“灰婴综合症”）。如在妊娠末期大量使用，可引起新生儿血小板减少症、再生障碍性贫血或胎儿死亡。甲砒霉素和氟甲砒霉素免疫抑制作用比氯霉素强且具有一定的繁殖毒性。

（2）四环素类抗生素：具有共同的基本母核氢化并四苯，仅取代基有所不同的一类化合物。它们的两性物质，通常在碱性水溶液中易降解，酸性水溶液中较稳定，故临床一般用其盐酸盐。常用的有四环素、土霉素、金霉素和强力霉素等。该类药物使用不当可致牙釉质形成不全，引起“四环素牙”，骨骼、心脏畸形，先

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