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An LC–MS/MS method for the determination of antibiotic residues in distillers grains

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ABSTRACT

Antibiotics are used in ethanol production to discourage the growth of bacteria that would result in lower ethanol content and a lower quality product. A survey conducted by the FDA (FY 2010 Nationwide Survey of Distillers Grains for Antibiotic Residues, 2009 [1]) revealed that the residues of these antibiotics can remain in the distillers grains (DG) by-product, which is used as an animal feed ingredient. The low levels of antibiotic residues in DG could be a public health concern, as they could lead to antimicrobial resistance. To enable the quantitative determination of these antibiotics (erythromycin, penicillin G, virginiamycin M1 and virginiamycin S1), we developed a sensitive LC–MS/MS method. The residues were extracted from distillers grains with a mixture of acetonitrile and buffer followed by acetonitrile. The combined extract was diluted with water and washed with hexane. An aliquot was cleaned up on an Oasis HLB solid phase extraction cartridge. Extracts were analyzed by LC-tandem mass spectrometry. The method was successfully validated using a variety of different matrices such as corn DG, corn & milo DG, and deoiled corn DG. Absolute recoveries of the analytes ranged from 53 to 106%. Accuracy ranged from 90 to 101% based on calibration by matrix standards. The limits of quantitation and relative standard deviation were all satisfactory to support future surveillance studies.

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1. Introduction

Distillers grains (DG) is a by-product of the fuel ethanol industry. During ethanol production, the starch from the grains is broken down and fermented into ethanol, while the other nutrients remain in the DG. This makes DG an attractive alternative feed ingredient because of its high content of energy, protein and minerals as well as its low cost in comparison to traditional sources of these nutrients [2]. In the USA, DG is commonly fed to beef and dairy cattle, swine, and poultry [3].

The production of DG has increased steadily over the past several decades. In 2015, more than 40 million metric tons of DG was produced in the USA [4]. There are two major milling methods—wet and dry milling—used to produce ethanol. The dry milling procedure produces dried distillers grains with solubles (DDGS); DDGS refers to the combination of the liquid and solid leftovers after the ethanol is removed. The liquid is concentrated by evaporation to form condensed distillers solubles [2], which is then added to the solid and mixed to produce DDGS. This process is more common than wet milling as DDGS is easier to store and transport. DG is predominantly sold commercially as DDGS. In this form, it is a stable, free-flowing granular product that is yellow/tan to brown in color with a bulk density of 30–40 pounds per cubic foot [5]. Increasingly, many ethanol plants are using a relatively new procedure to extract part of the corn oil from DG, which results in DDGS with low oil content commonly referred to as deoiled DDGS. DDGS contains about 11–13% crude fat while the deoiled variety contains about 7–8% [6]. The extracted oil is marketed for biodiesel or feeding to other livestock [6].

During fermentation, antibiotics are added to combat bacterial contamination that could result in lower ethanol yield and quality [2]. However, this antibiotic use can lead to residues in the DG that is fed to animals intended for human consumption. FDA's Center for Veterinary Medicine (CVM), which has the regulatory authority for all drugs, additives, and ingredients used in animal feeds, conducted a nationwide survey in 2010 to detect possible antibiotic residues in DG [1]. The survey found erythromycin (ERY), penicillin G (PEN G) and virginiamycin M1 (VIR M1) to be present in some samples. The use of DG with low levels of antibiotic residues as feed could be a public health concern because of its potential to lead to the development of bacterial resistance. To be able to quantify the antibiotic drugs at low levels, CVM needed a sensitive analytical method.

We previously developed an LC-tandem mass spectrometry method [7,8] which was used to carry out the 2010 survey of

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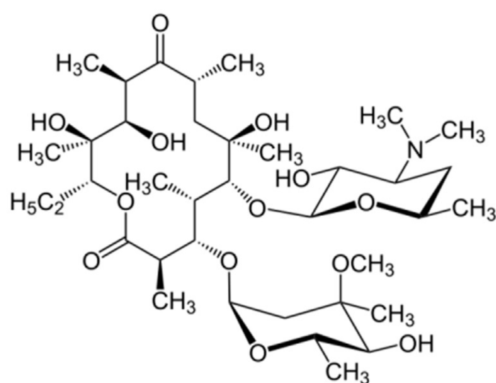
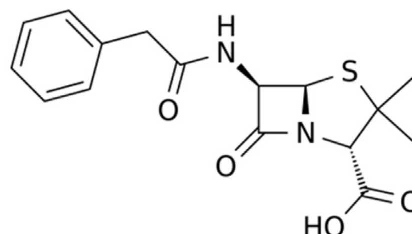


Erythromycin A



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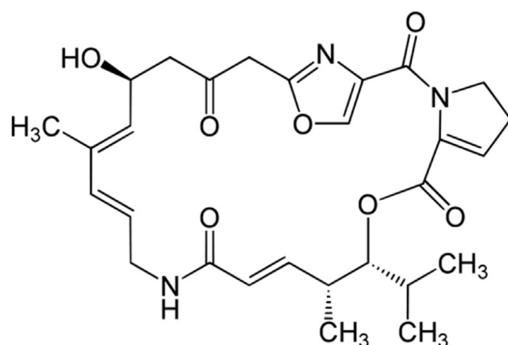
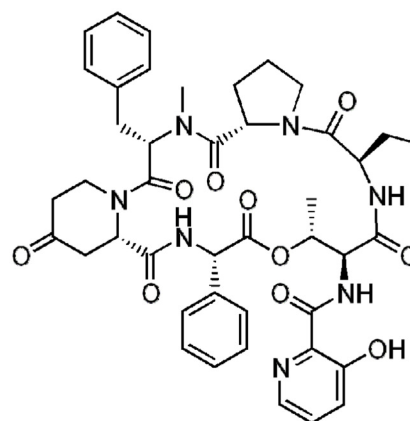


Virginiamycin M1



Virginiamycin S1

Fig. 1. Structures of target compounds.

antibiotics in DG [1]. However, this method used an ion-trap mass spectrometer, which was not adequately sensitive to measure low antibiotic levels required in the current setting. Hence, in this study, we developed a more sensitive quantitative LC–MS/MS method that used a triple quadrupole mass spectrometer. Additionally, as only four analytes—ERY, PEN G, VIR M1 and virginiamycin S1 (VIR S1) (Fig. 1)—were included in this method, there was more latitude for method optimization for individual analytes in comparison to the previous method with thirteen analytes of diverse chemistries.

2. Materials and methods

2.1. Materials

Antibiotic standards, ERY A, PEN G, VIR M1, VIR S1 and erythromycin-(*N*-methyl-^{13}C,d$_3$) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Benzyl penicillinate-d$_7$ was purchased from Toronto Research Chemical, Inc. (Toronto, ON, Canada).

Ethanol (99.9%) and formic acid (95%) were obtained from Sigma-Aldrich, Inc. Acetonitrile (HPLC grade) was purchased from Burdick & Jackson (Muskegon, MI, USA). Sodium acetate trihydrate (99.5%), glacial acetic acid, and hexane (HPLC grade) were purchased from Fisher Scientific (Pittsburgh, PA, USA). Water was purified with a Milli-Q system (Millipore, 18.2 MΩ cm at 25 °C). Oasis HLB 60 mg/3 mL cartridges used for solid phase extraction (SPE) were obtained from Waters Corporation (Milford, MA, USA).

Distillers grain samples used in the method development and validation were provided by several commercial ethanol distillation plants.

Acetate buffer (1 M, pH 5.0) was prepared by dissolving 50 g of sodium acetate trihydrate in 18 mL of glacial acetic acid with water to a total volume of 500 mL. If needed, pH was adjusted using either acid or salt.

2.2. Preparation of standard solutions

Approximately 10 mg of each drug (ERY, PEN G, VIR M1 and VIR S1) was weighed, transferred to 100 mL volumetric flasks, and dissolved in the appropriate solvent (water for PEN G and acetonitrile for the others) to prepare about 100 μg/mL individual stock standards. The concentration of each standard was calculated by correcting for purity and salt content. A 25 μg/mL mixed standard was prepared by transferring a volume equivalent to 500 μg

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