Determination of Tetracycline Antibiotics in Beef Tissues Using Ion-Paired Liquid Chromatography

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ABSTRACT

Beef samples were obtained from local butchers and Whole Foods (an organic market) in order to determine and compare the amount of Tetracycline antibiotics in organic and non-organic beef. Extraction was used to separate the antibiotics from the meat tissues and then High Powered Liquid Chromatography (HPLC) was used to analyze the amount of antibiotics in the samples. Currently, many farm animals are feed antibiotics in order to provide therapeutic treatment. Even though, antibiotics are not toxic, they can cause some harm to consumers who eat meat. In this study, the amount of antibiotics in organic and non-organic meats will be determined and compared.

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INTRODUCTION

Tetracycline is an antibiotic which is used widely in humans and animals. Tetracycline also known as TC's, are characterized by their exceptional chemotherapeutic efficiency against a wide range of bacteria. Tetracycline is used in the cattle industry to promote growth and may result in the presence of tetracycline residues in edible animal tissues. Residue in edible meat tissues can result in allergic reaction, as well as increased antibiotic resistance, which is a growing concern in the medical field. Although TC's are not considered toxic, the United States have recently increased their levels to 2 ppm in muscle, 6 ppm in liver, and 12 ppm in kidney.

We will be conducting an experiment which will conclude the amount of tetracycline in beef samples from different places. In recent years there have been several studies determining tetracycline in meat, and an accurate and precise method is important. HPLC or high powered liquid chromatography is a widely used technique which separates and helps identify biological substances, and can be used to determine tetracycline in meat.

Figure 1. Beef Liver Samples

RESULTS

According to the experimental article, if Tetracycline is present in the sample, we should see a peak around 8 to 10 minutes. Also, in the graph for the standard (1000 ppm), we saw a peak around 9 minutes for the Tetracycline (Graph 1). Since, the beef sample showed no peak around 8 to 10 minutes, we concluded that there was not Tetracycline present in this particular sample (Graph 2).

METHODS AND MATERIALS

A modified version of the *Determination of Tetracycline Antibiotics in Beef and Pork Tissues Using Ion-Paired Liquid Chromatography* was used in this experiment.

The Extraction Method:

The beef liver (non-organic) was blended and 45 g was weighed out. Deionized water (3mL/g) was added to the blended mixture and mixed again for 2 minutes. 12 mL of homogenate sample was transferred into a 125 mL conical flask, and then 48 mL of Acetonitrile was added to the sample and mixed thoroughly. 3 mL of 0.1 M phosphoric acid (H_3PO_4) was added and the mixture was allowed to stay for 15 minutes. The supernatant was decanted by pipetting out 12 mL of the filtrate and filtering through filter paper.

The Evaporative Concentration Method:

The filtrate was transferred to a 125 mL separatory funnel and then 10 mL of Hexane and 10 mL Dichloromethane were added to the funnel (Figure 2). The mixture was shaken until distinct layers were observed. The lower water layer was collected in a 15 mL graduated conical centrifuged tube. The organic layer in the separatory funnel was washed with .5 mL of water and the washings were combined with the other water layer. The organic layer was then discarded. The filtrate was placed in a 15 mL vacuum flask, it was attached to the vacuum and it was warmed on a hot plate. The vacuum and the heat were applied for 15 minutes and the contents were not allowed to boil out. The contents were evaporated to less than 1 or 2 mL. The contents were filtered through a filter cartridge into a test tube.

HPLC Method:

The mobile phase mixture of 4mM Oxalic Acid, 4mM sodium oxalate and 4mM Sodium Decane Sulfonate and water and Acetonitrile (700mL:300mL) was used. Then, HPLC analysis was performed using an Atlantis T3 5 µm 4.6*150 mm column (Figure 3).

Figure 2. Seperatory Funnel Extraction

Figure 3. Atlantis T3 5 µm column

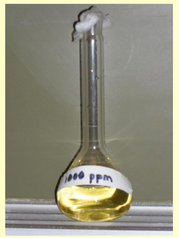
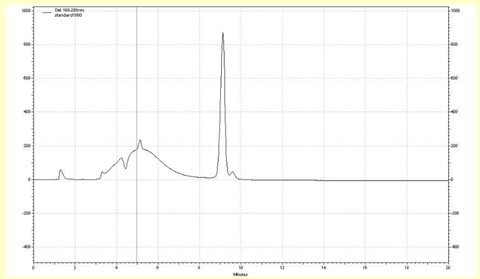
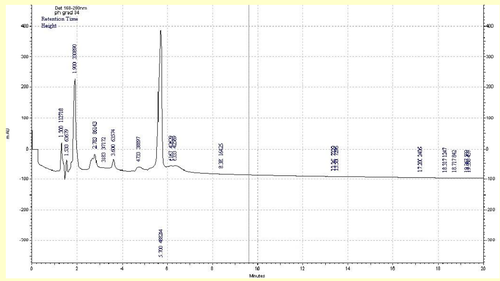


Figure 4. Standard 1000ppm



Graph 1. Standard 1000 ppm



Graph 2. Beef Sample

DISCUSSION

Due to time restraints and instrumental problems, we were unable to determine clear results for our study concerning the amount of tetracycline in organic and non-organic beef. The graphs that were obtained allowed us to determine that there probably is not any antibiotics in the beef sample. Since we were only able to test one sample, we could not compare our graph to that of any other beef liver sample(organic and non-organic). Also, the data that we obtained was not completely comparable to the experimental research article because we did not use the same column (Figure 3).

CONCLUSION

Overall, the results were inconclusive since we were unable to determine and compare, the amount of tetracycline antibiotics in organic and non-organic beef samples. The procedure will be repeated again for the organic and non-organic beef samples. In the future, we can also use this experiment to determine the amount of Tetracycline in pork and chicken tissues.

REFERENCES

American Chemical Society. *Determination of Tetracycline Antibiotics in Beef and Pork Tissues Using Ion-Paired Liquid Chromatography*. 2000. http://pubs.acs.org/doi/abs/10.1021/jf990649r

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