

Abstract

Throughout the U.S., there are 961 types of dog foods. Many include preservatives; however, one has gained notable attention for the hazards that it poses to our canine friends. Butylated hydroxytoluene (BHT) is an historic-use preservative, which is now known to be a carcinogen, and tumor-growth promoter. While its use has not been outlawed, many brands have turned to other preservatives, in light of recent findings. Throughout the industry, however, no dog foods publicize the use of BHT, so that its remaining use is unknown. In this research, three common brands of dog food, Acana, Wilderness, and Wellness, were analyzed for their BHT content, to shed light on BHT prevalence in artificially-formulated dog food. For each, 10g of hard dog food as to a powder, and soaked in 20 ml of ethanol for 24h. For each dog food ethanol filtrate, 10µl was placed on an FTIR spectrometer Diamond ATR accessory, and dried to a thin-film. ATR-FTIR analyses of the three foods suggested the presence of BHT in the extract, along with other ethanol-soluble components. To better understand BHT content, HPLC was used, with a mobile phase of Acetonitrile and 99%Water/1%Acetic Acid, and 280nm detection. BHT was successfully located at 3.3 minutes retention time, and serial dilutions of a 0.66mg/ml BHT in ethanol were made, to create an HPLC calibration. HPLC analyses of dog-food ethanol extracts highlight BHT presence in all foods tested, with 0.124% BHT in Wilderness, 0.104% BHT in Wellness, and 0.101% BHT in Acana dog food. These BHT levels are 50-62 times the recommended safety levels.

BHT in Dog Food

- There are over 961 dog food brands here in the US. However, not everyone knows about the carcinogen chemical located within them. This chemical is known as Butylated Hydroxytoluene, or BHT.
- Butylated Hydroxytoluene is an antioxidant used in raw materials. BHT has been added as a preservative in dog food since 1940. BHT is found in many common types of dog foods that are currently available to the consumer, and is also used as a preservative for cosmetic items, and many types of fuels. As a preservative, BHT keeps dog food fresher, and free of bacterial contamination, thus extending the self-life of the product, to thereby extend the "sell-by" date, and storage of the dog food once at the consumer's home.



Images courtesy of the respective manufacturer.

Hazards of BHT

- Recent research has suggested that BHT poses a significant health risk to pets when used in dog food, and humans, when found in cosmetics. For dog foods in particular, researchers have found that preservative concentrations of BHT may lead to cancer, liver damage and respiratory problems for our beloved pets.
- Findings from the National Library of Medicine, using rats as model pets, demonstrated that BHT at lower doses produced increased liver weight, and decreased activity of several hepatic enzymes. In addition to liver and kidney effects, BHT applied to the skin was associated with toxic effects in lung tissue, blood coagulation, and was a tumor promoter in some cases.
- In spite of these warnings, the cheapest to most expensive brands of dog food, whether advertised as natural, organic, or artificially formulated, may contain this carcinogen, without labeled warning to the consumer.
- To tackle this problem, the Panel on Additives and Products or Substances used in Animal Feeds, (or FEEDAP) has provided guidelines for BHT content for dog foods. They suggest that BHT must remain under 150 mg or 0.002% of overall food content, to be considered safe for the animal.
- Unfortunately, many dog food manufacturers surpass this warning concentration in the production of their dog food, to extend the storage time of their products during the "factory production to store shelf" cycle.

Engineering Goal

This research seeks to highlight the prevalence of Butylated Hydroxytoluene use in the most common and most expensive dog foods. This research will broaden the idea of not using BHT within dog foods.

Extracting BHT from Dog Food

- For each dog food to be tested, 10 grams of hard dog food was ground to a powder using a coffee grinder.
- The powder was then submerged in 20 ml of ethanol for 24 hours. The reason ethanol is used is because Butylated Hydroxytoluene is insoluble in water. If the ground dog food were dissolved in water, little/no BHT would move to the water, and be detected.
- After 24 hours of mixing in ethanol, the solid dog food powder remnants were then filtered and separated from the ethanol solvent, which likely contained BHT.

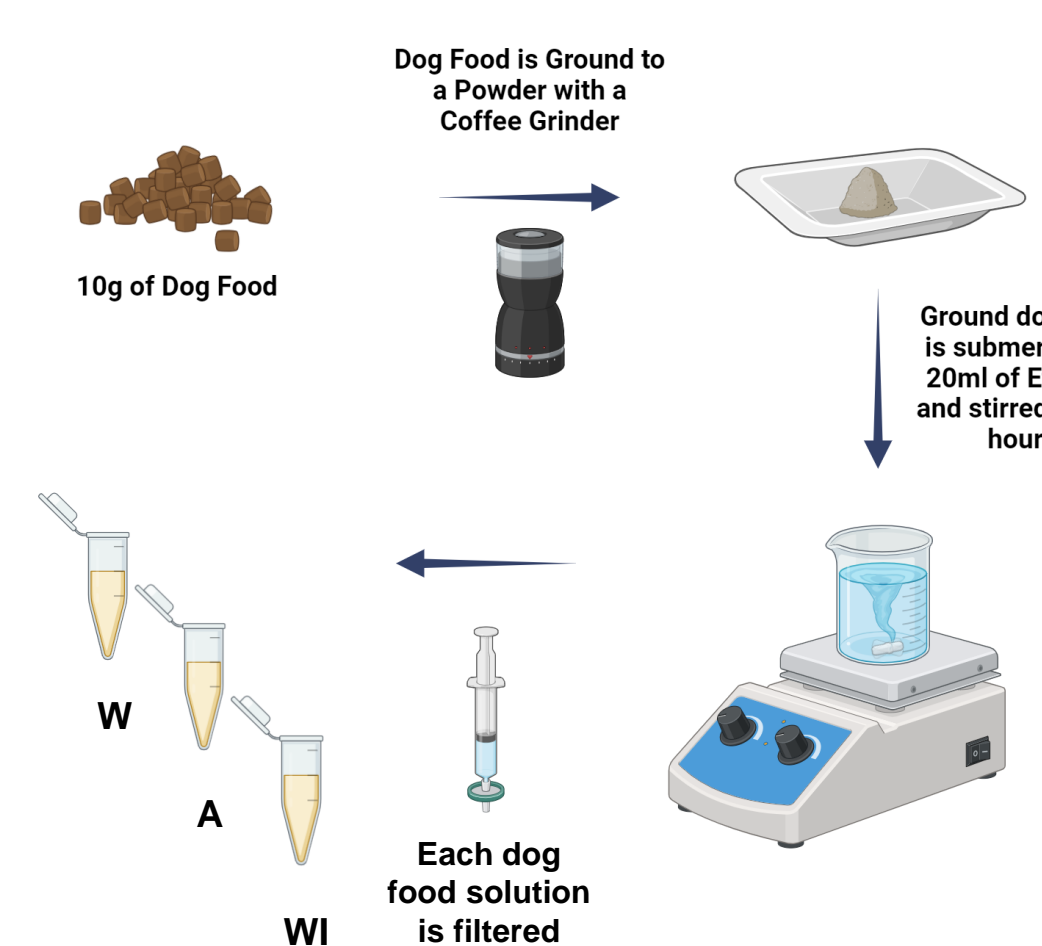


Figure 1. Extraction of BHT from Dog Food

Unless otherwise noted, all images and graphs were created by the student researcher.

Shedding Light on the Prevalence of Harmful Butylated Hydroxytoluene Preservative in Artificially Formulated Dog Foods

ATR-FTIR Spectra of Dog Foods

For each dog food ethanol extract, 10µl of the sample was placed atop the Attenuated Total Reflectance (ATR) Fourier Transform Infrared (FTIR) spectrometer diamond crystal, and permitted to air dry, to obtain an FTIR spectrum of the dried thin film, which provided insight about the chemical composition of what was extracted from the hard dog food. For each food, the spectrum of the ethanol-extract was compared to an ATR-FTIR spectrum of ~2mg of BHT, similarly measured using the ATR-FTIR. These comparisons are shown in Figures 2-4 for Wellness, Wilderness, and Acana Dog Foods, respectively.

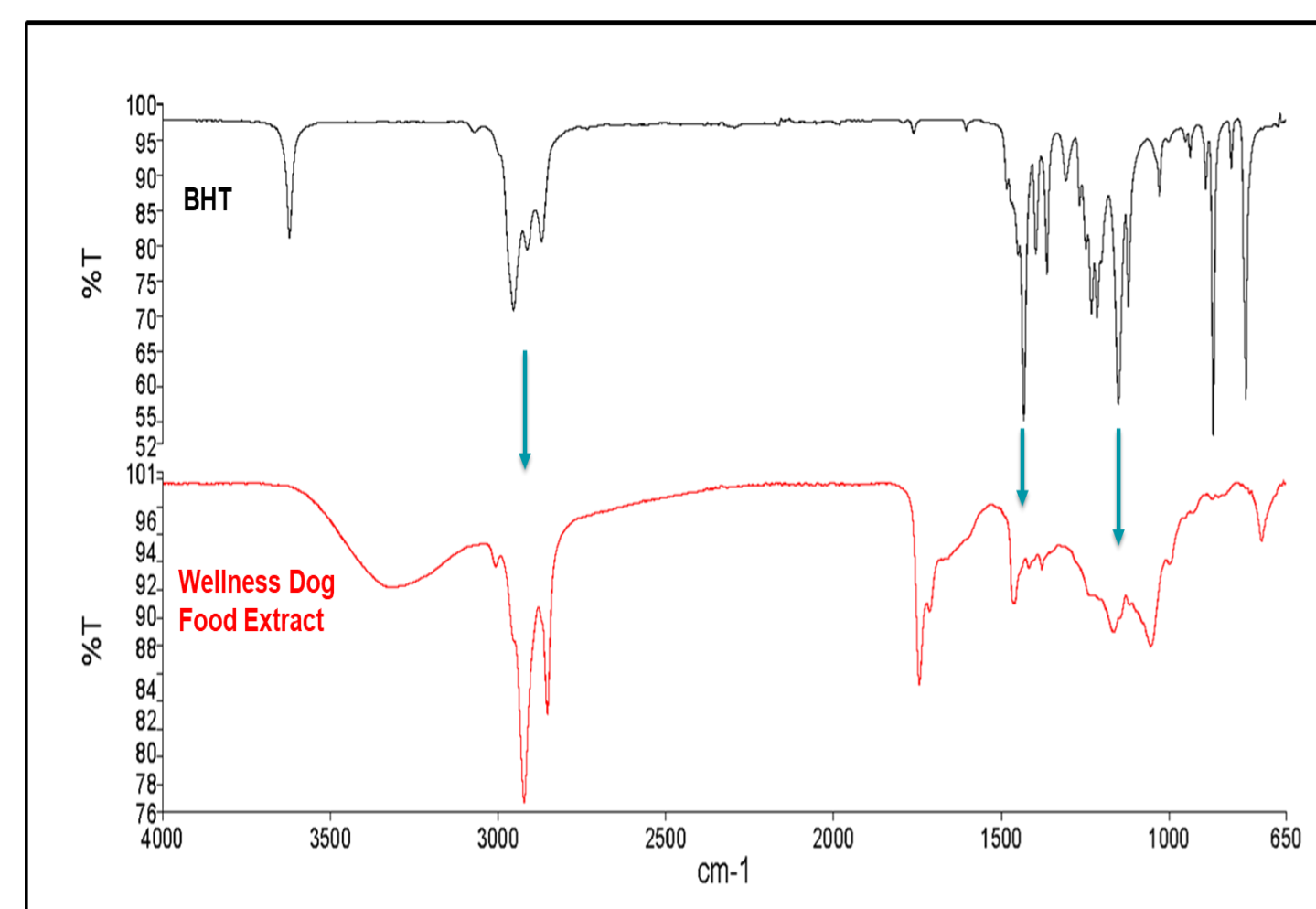


Figure 2. The ATR-FTIR spectrum of the Wellness Ethanol Extract, versus the ATR-FTIR spectrum of BHT highlights the presence of the BHT preservative amongst other ethanol soluble dog food components.

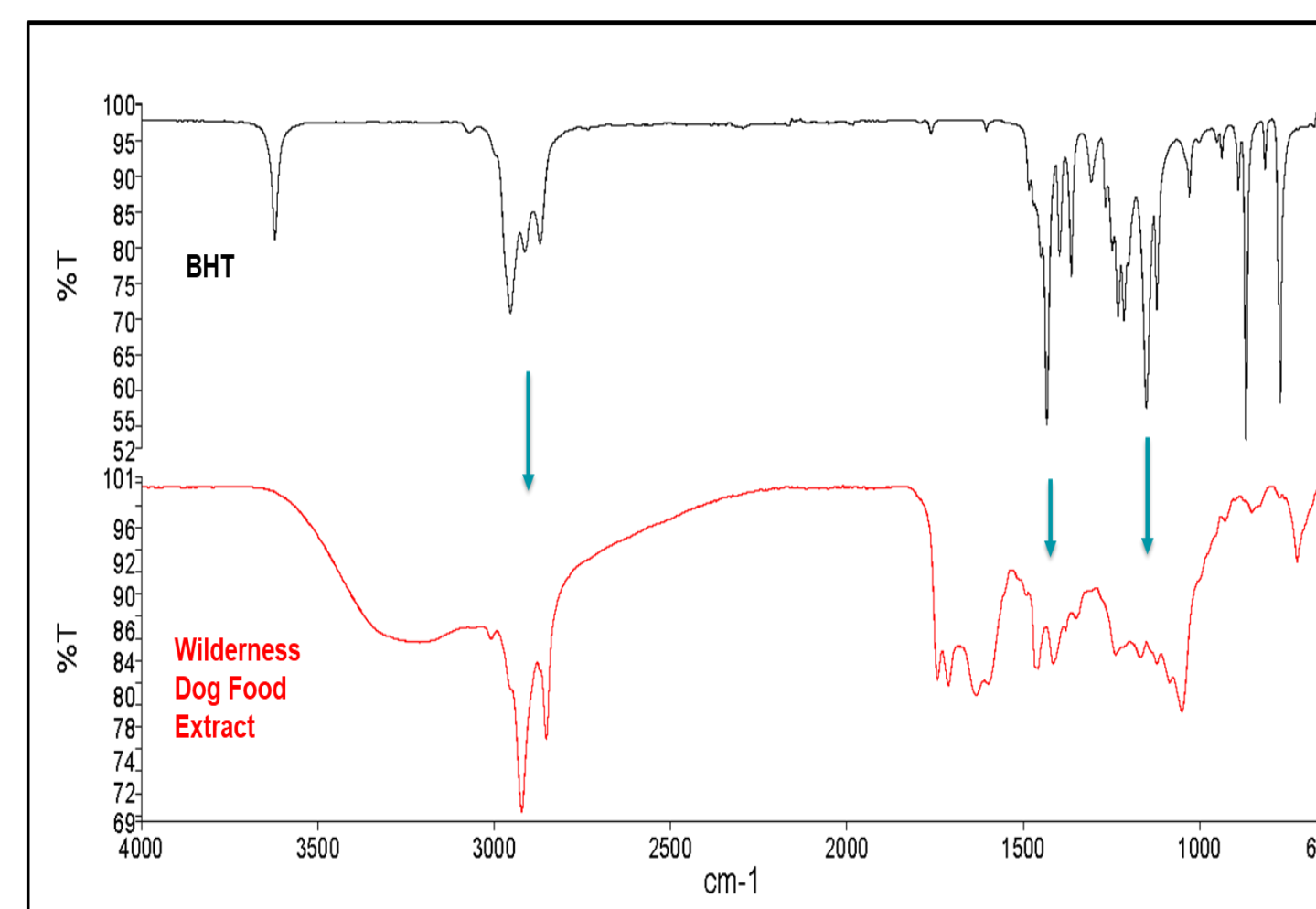


Figure 3. The ATR-FTIR spectrum of the Wilderness Ethanol Extract, versus the ATR-FTIR spectrum of BHT highlights the presence of the BHT preservative amongst other ethanol soluble dog food components.

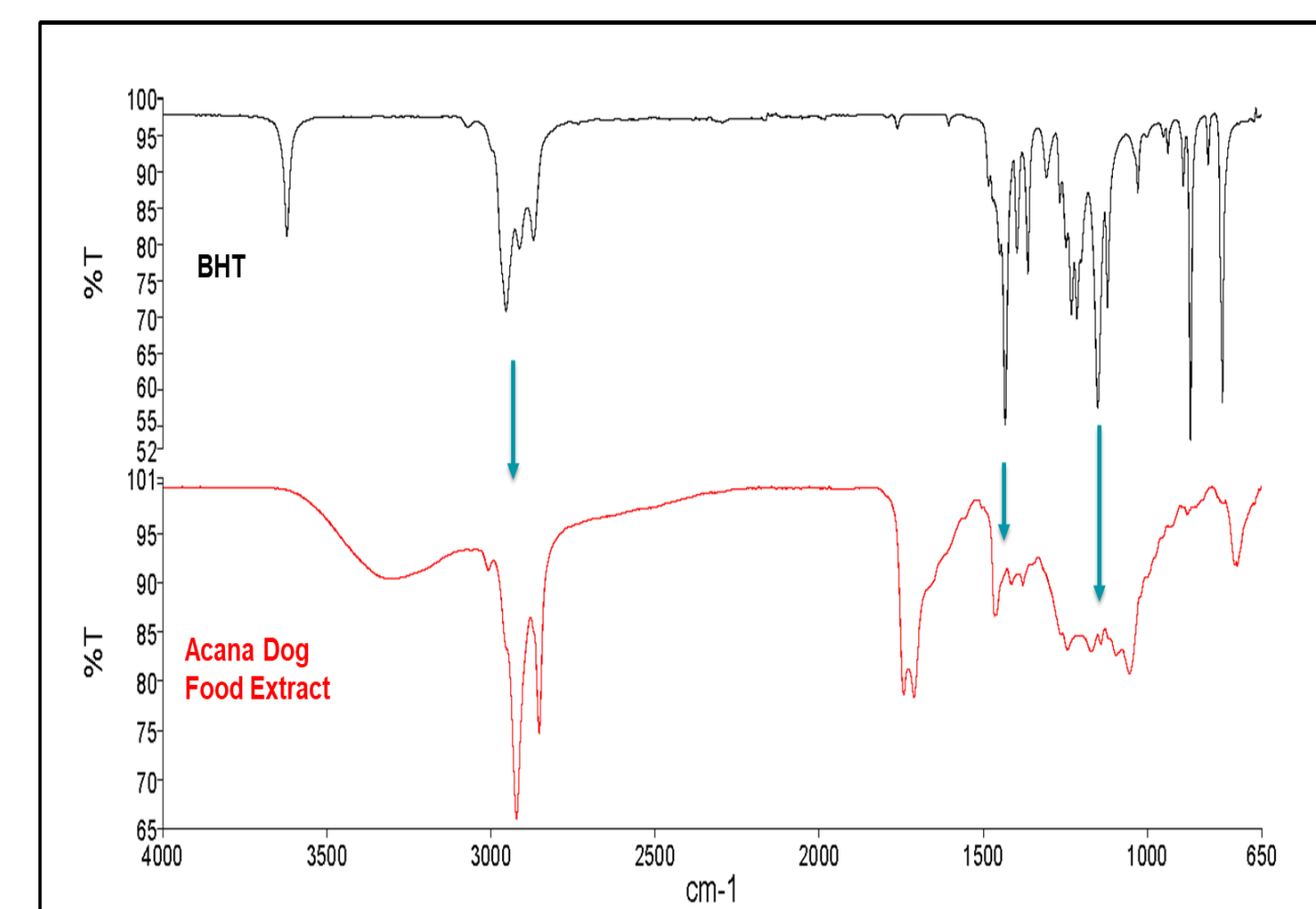


Figure 4. The ATR-FTIR spectrum of the Acana Ethanol Extract, versus the ATR-FTIR spectrum of BHT highlights the presence of the BHT preservative amongst other ethanol soluble dog food components.

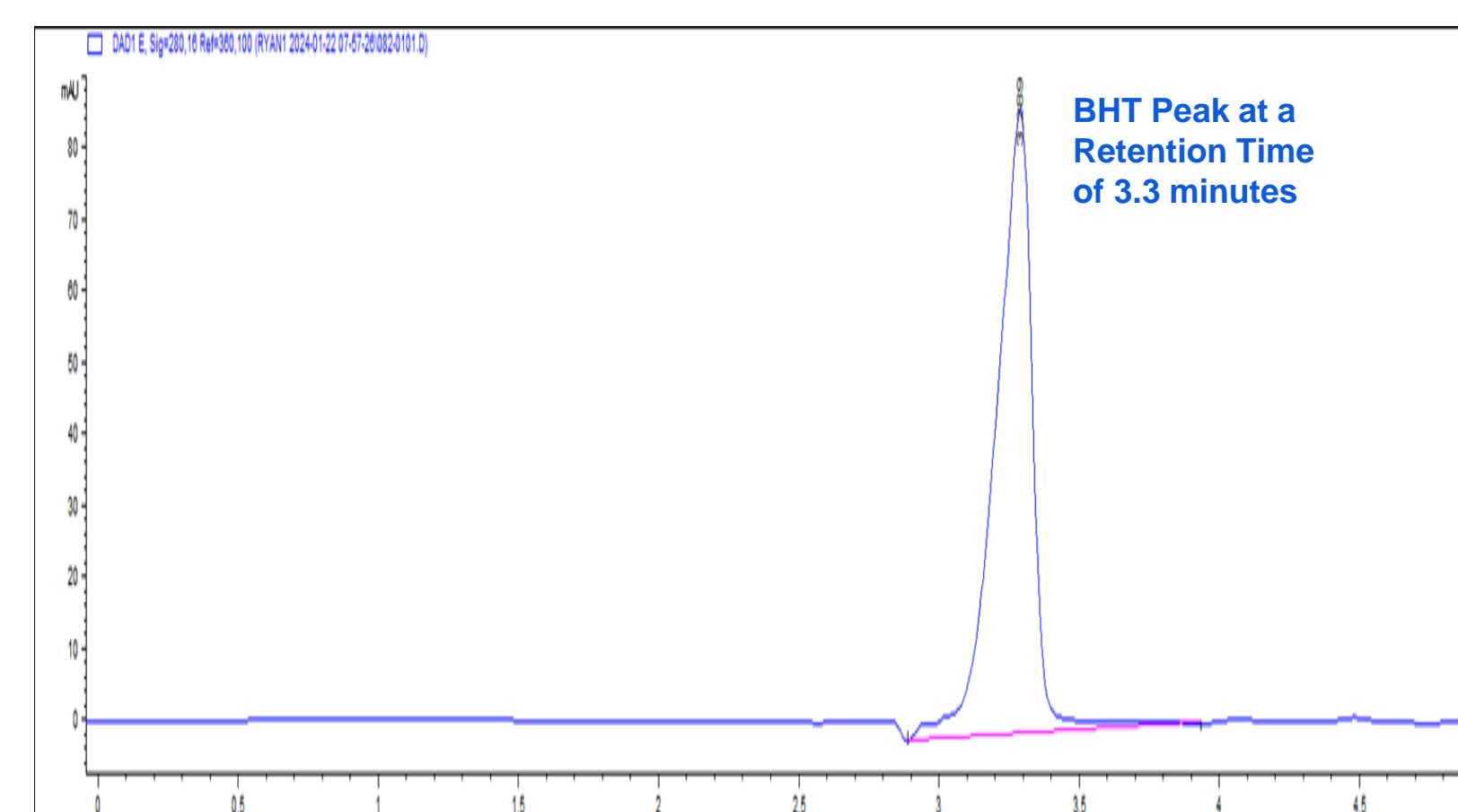
Comparison of each dog food ethanol extract ATR-FTIR spectrum with that of BHT suggests that BHT is present in each of the dog foods tested, due to the presence of spectral peaks that are in common with BHT (blue arrows in Figs. 2-4). However, due to the overlapping of spectral features from other dog food components that were extracted by the ethanol solvent it was hard to prove it was found in the solution. As such, further analyses were needed, using high pressure liquid chromatography, where each ethanolic extract mixture was separated, and BHT could be detected and quantified.

Analysis of Dog Food Extracts by High Pressure Liquid Chromatography

According to literature by Ahmad, Reversed Phase HPLC can be used to separate and detect phenolic compounds, including BHT, found in personal care products. This same methodology will be adapted for use in detecting BHT in ethanol-extracts from dog food, using the following HPLC method parameters shown below. Using a 0.66mg/ml sample of BHT in ethanol, a peak for BHT was measured at a retention time of 3.3 minutes (Fig. 5). This peak will be used to identify BHT in the dog food extracts.

- Mobile Phase:** Solvent A: Acetonitrile, Solvent B: 99%Water-1% Acetic Acid
- Column Temperature:** 25°C
- Injection Volume:** 5µl
- Flow Rate:** 1.0 ml/min
- UV-Vis Detector:** 280nm, with Reference Wavelength of 360nm
- Column:** RP-C18 Metacil (5µm) ODS column, 4.6 mm x 250 mm

Figure 5. The HPLC chromatogram of the 0.66mg/ml BHT in ethanol highlights a BHT peak with a retention time of 3.3 minutes, and corresponding peak area of 308mAu*sec. (Inset: the student researcher working at the Agilent 1100 HPLC)



Detection of BHT in Ethanol Extracts of Consumer Dog Food using HPLC

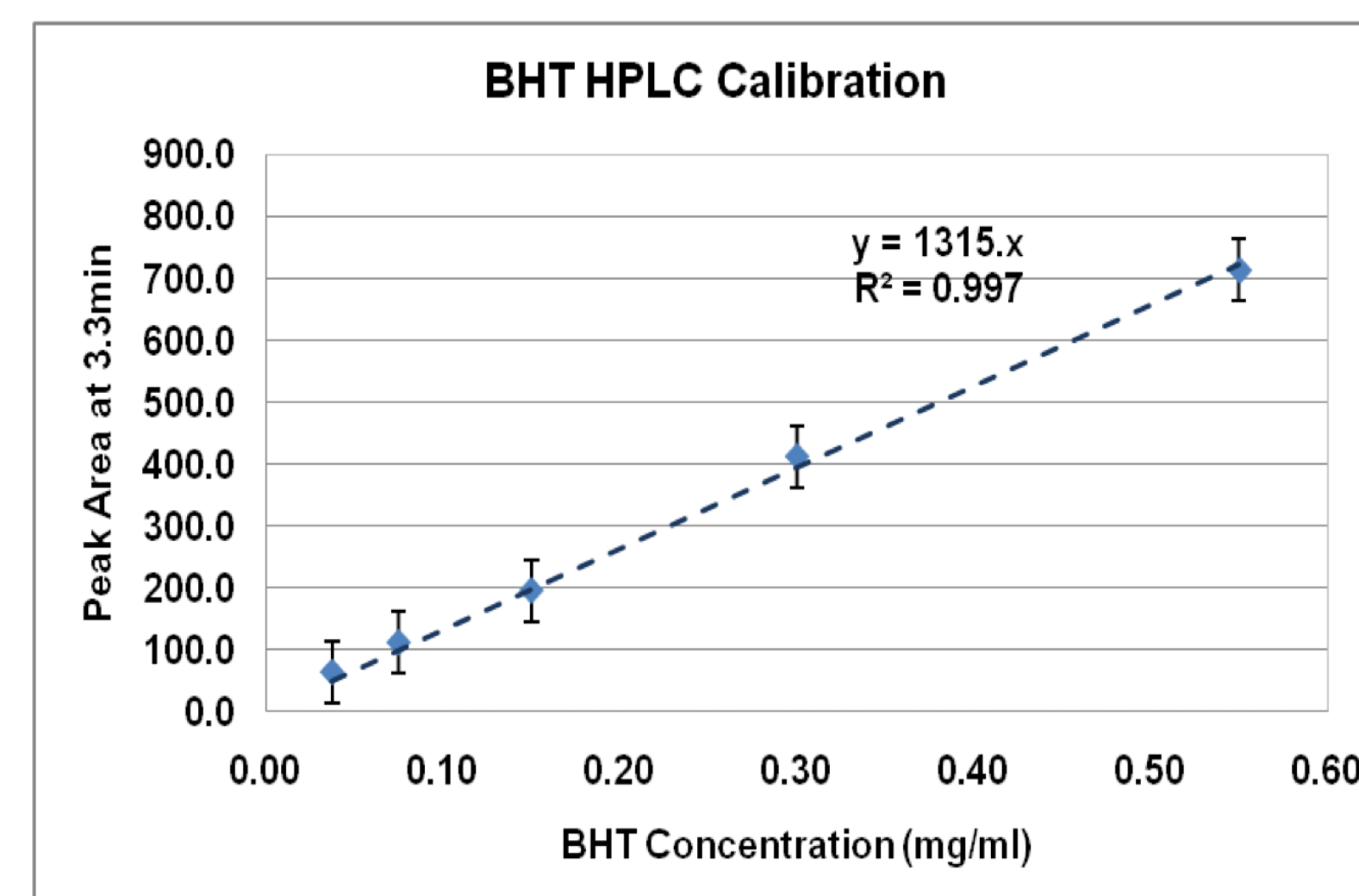
In order to quantify the amount of BHT in each of the dog food extracts, a HPLC calibration was performed, so that instrument response, or peak area, could be converted to amount of BHT.

- To create such a calibration, calibration standards of 0.55, 0.30, 0.15, 0.08, and 0.04 mg/ml BHT in reagent grade ethanol were created.
- Each of these calibrations standards were pipetted into autosampler vials, and the chromatogram for each standard was measured.
- The peak area at 3.3 minutes for each calibration standard was determined using the HPLC software (Table 1).
- Then, the concentrations of BHT (in mg/ml) were plotted against their respective peak areas at 3.3 minutes, to create the HPLC Calibration for BHT, shown in Figure 6.

Table 1. HPLC Calibration Data, including calibration standard BHT concentrations, and resulting peak areas at 3.3 minutes

Concentration (mg/ml)	Average Peak Area at 1.6 min
0.55	712.8
0.30	411.4
0.15	194.3
0.08	110.0
0.04	62.1

Figure 6 (right). The BHT Concentration for each calibration standard is plotted against its peak area (at 3.3 mins), and a linear, best fit Calibration plot was created.



Detection of BHT in Ethanol Extracts of Consumer Dog Food using HPLC

- For each dog food to be evaluated (Wellness, Acana, and Wilderness), the separate ethanol extracts were filtered to remove solids, and each were evaluated via HPLC, using the same method from Figures 5 and 6.
- This will allow me to determine the BHT inside each of the dog food extracts, and eventually, the %BHT in each food product.
- The HPLC chromatogram for Wilderness dog food is shown to the right in Figure 7. Careful inspection highlights a peak at 3.3 minutes, which is due to the presence of BHT. For Wilderness dog food extract, the calculated peak area was 813.98.

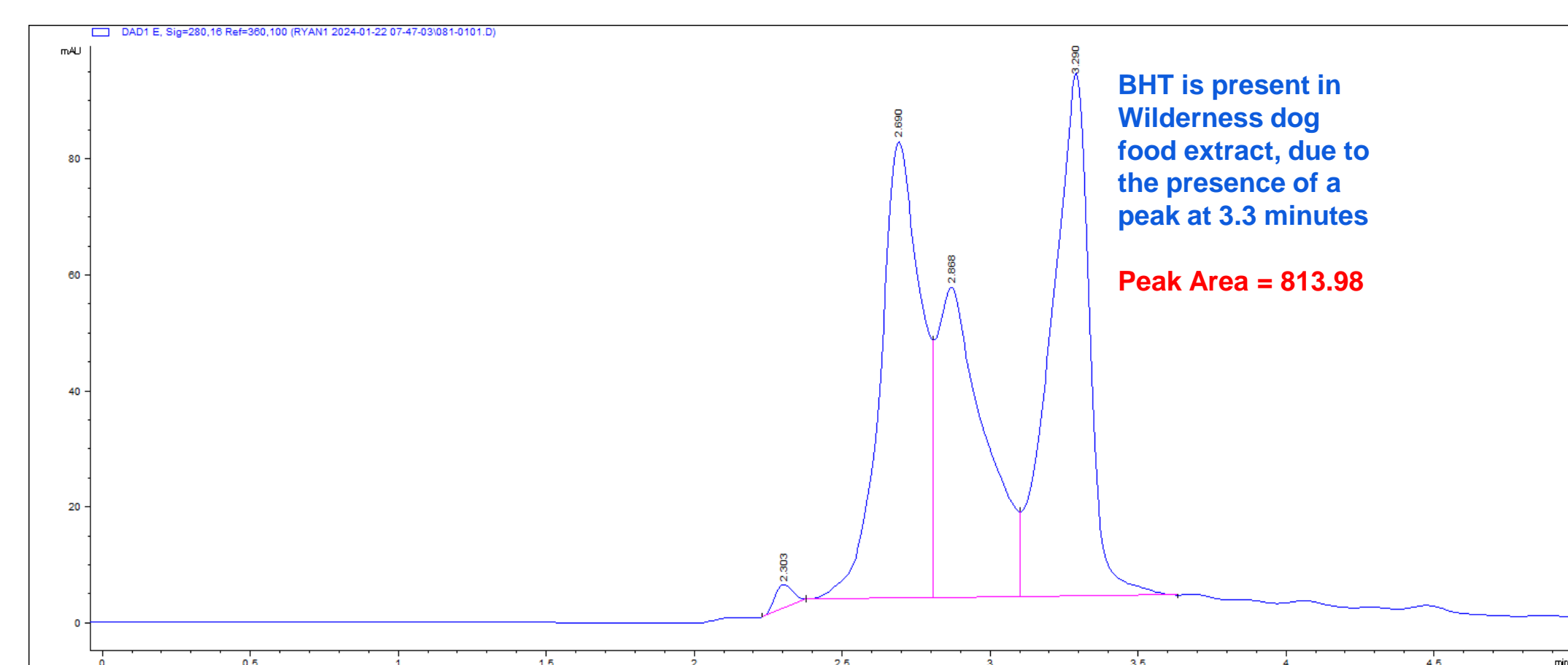


Figure 7. HPLC chromatography of Wilderness dog food

HPLC of Dog Food Extracts

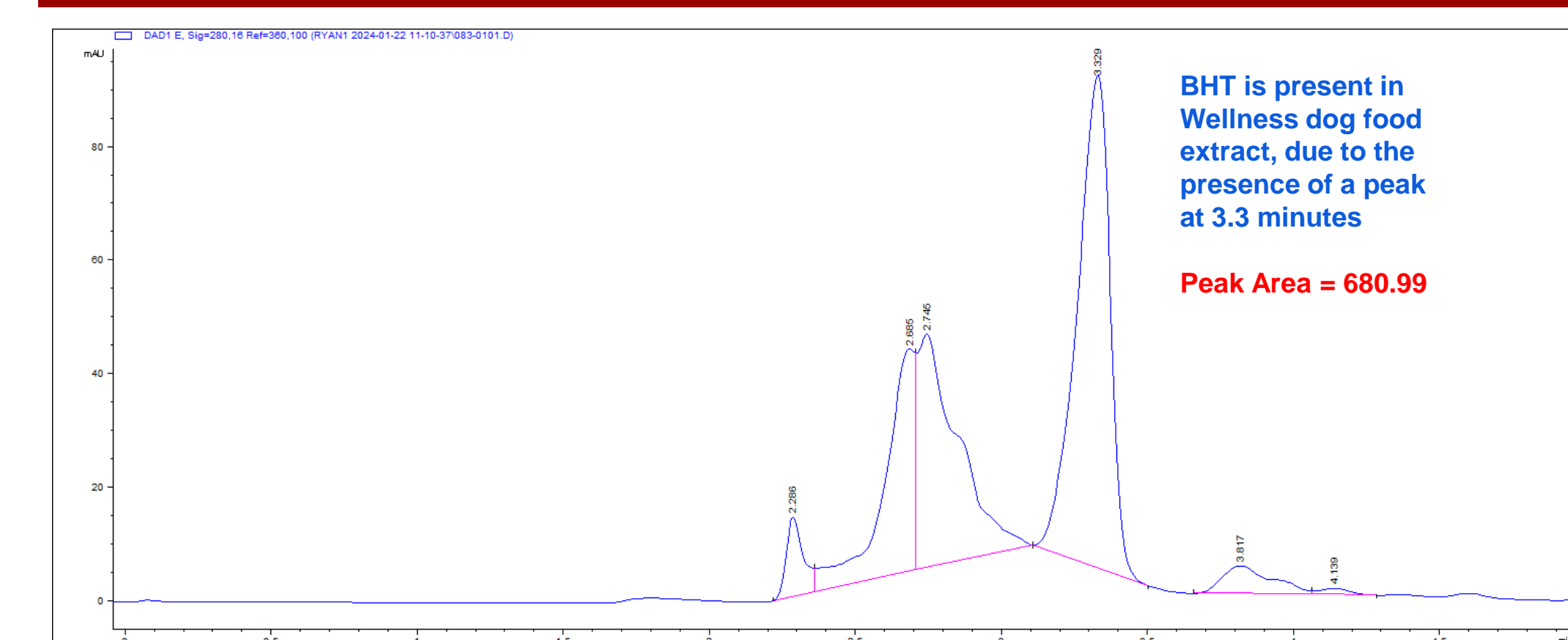


Figure 8. HPLC chromatography of Wellness Dog Food

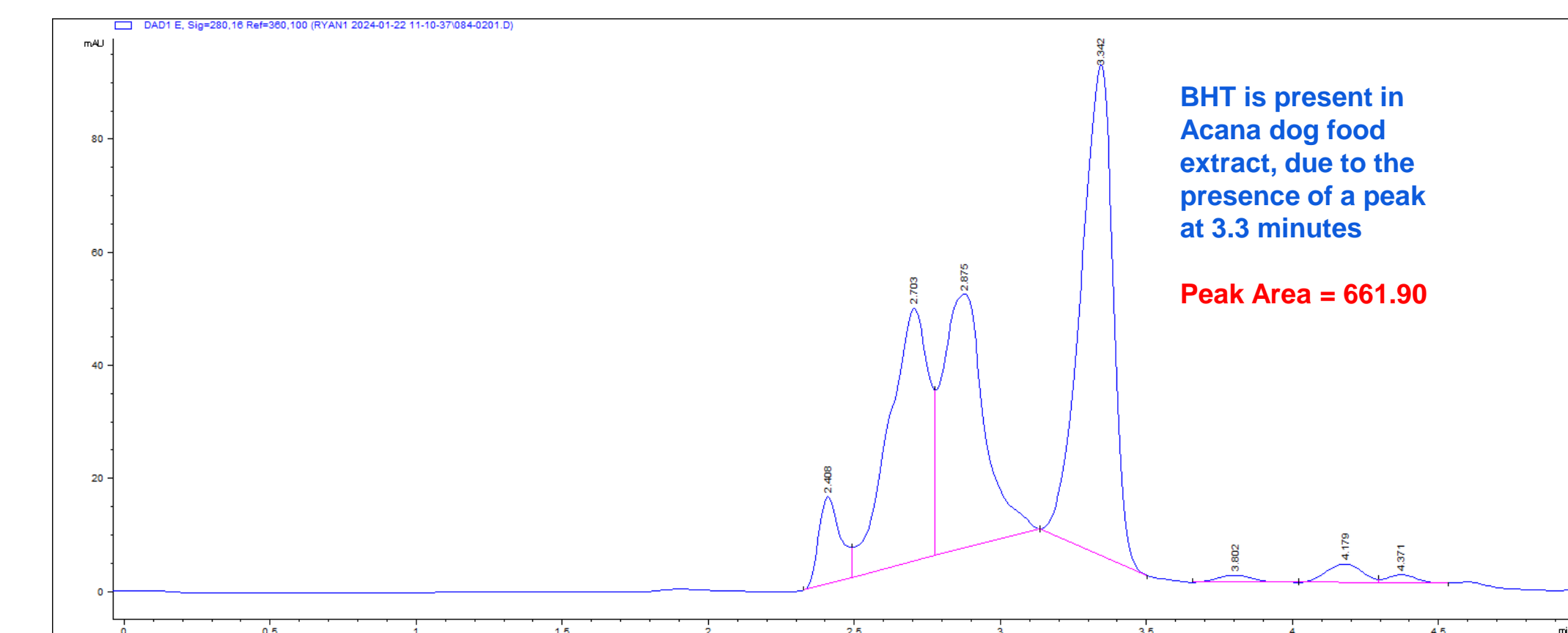


Figure 9. HPLC chromatography of Acana Dog Food

Similarly, the HPLC chromatograms for Wellness and Acana dog foods are shown in Figures 8 & 9. BHT peak areas at 3.3 minutes for Wellness and Acana dog foods were 680.99 & 661.90, respectively.

Calculations

- HPLC peak areas at 3.3 minutes for each of the dog food extracts (from Figures 7-9, and tabulated in Table 2, column 2) were converted to concentration of BHT (in mg/ml) for each dog food brand.

$$\text{Conc. of BHT (mg/ml)} = \text{Peak Area at 3.3min} / \text{Slope from BHT Calibration}$$

i.e. For Wilderness Dog Food

$$\text{Wilderness Conc. of BHT (mg/ml)} = 813.98/1315 = 0.62 \text{ mg-BHT/ml}$$

- These values were multiplied by 20ml, to obtain the mg of BHT, which was converted to grams of BHT (column 4). Each BHT mass (in grams) was divided by 10g (the original mass of dog food that was soaked in ethanol), to obtain the %BHT (column 5). Results indicate highlight %BHT content of 0.101-0.125%, which is far more than the 0.002% recommended safety content for BHT.

$$\text{Wilderness Mass of BHT (grams)} = 0.62 \text{ mg} * 20\text{ml} * (1\text{g}/1000\text{mg}) = 0.012\text{g}$$

$$\text{Wilderness \%BHT} = (0.012\text{g}/10\text{g original mass}) * 100\% = 0.124\%$$

Table 2. Calculations from dog food extract peak area to %BHT content.

Dog Food	Peak Area at 3.3 min	Concentration of BHT in mg/ml (Peak Area/1315)	Mass (in grams) of BHT in Dog Food (BHT Conc * 20ml)	%BHT in Dog Food (Mass of BHT in Dog Food/Original Dog Food Mass of 10g)
Wilderness	813.98	0.62	0.012	0.124%
Wellness	680.99	0.52	0.010	0.104%
Acana	661.90	0.50	0.010	0.101%

Discussion & Conclusions

- BHT can cause serious harm to dogs, and has been shown to cause cancer, and promote tumor growth.
- Wilderness, Acana, Wellness Dog Foods, each labeled as "organic," which implies that they are fresh and healthy for dogs, each contain more than the recommended "safe" concentration of BHT, which is 0.002%.
- Each manufacturer failed to share unsafe levels of BHT with pet owners.
- Wilderness includes 0.124% of BHT as a preservative, which is 62x the recommended safety level.
- Wellness includes 0.104% of BHT as a preservative, which is >50x the recommended safety level.
- Acana includes 0.101% of BHT as a preservative, which is >50x the recommended safety level.

Future Research

Further research would explore heather preservatives that could be used in dog food, For example vitamins that can extend the shelf life of the dog food that would pose less risk and harm to the consumer. Future research would also explore more enforcement of information on labels. The reason being is these brands did not mention any information of BHT being used. Having them add everything they used would give consumers more options and details when buying the specific brand.

Select References

- Akkbik, M., et al. Optimization and Validation of RP-HPLC-UV/Vis Method for Determination Phenolic Compounds in Several Personal Care Products. *International journal of analytical chemistry*. 2011. 858153. 10.1155/2011/858153.
- "Safety and efficacy of a feed additive consisting of butylated hydroxytoluene (BHT) for all animal species (Katyon Technologies Limited)." NCBI, 10 May 2022, Suzuki, David. "The Dirty Dozen: BHA and BHT." David Suzuki Foundation.
- "2,6-di-tert-butyl-p-cresol (BHT) CAS N°:128-37-0." OECD Existing Chemicals Database, 12 January 2004.