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Benzotriazole and Tolytriazole Analysis in Select Surface Waters near Wilmington Air Park

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BENZOTRIAZOLE AND TOLYTRIAZOLE ANALYSIS IN SELECT SURFACE WATERS NEAR WILMINGTON AIR PARK

A thesis submitted in partial fulfillment of the

requirements for the degree of Master of Science

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B.S., Wright State University, 2019

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY Lee A. Raska ENTITLED Benzotriazole and Tolytriazole Analysis in Select Surface Waters near Wilmington Air Park BE ACCEPTED IN PARTIAL FULFULLMENT OF THE REQUIREMENTS FOR THE DEGREE OF Master of Science.

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ABSTRACT

Raska, Lee A. M.S., Department of Chemistry, Wright State University, 2021. Benzotriazole and Tolytriazole Analysis in Select Surface Waters near Wilmington Air Park.

Previous investigations into the presence of benzotriazole (BTZ) and corresponding analogs done in early 2019 found elevated levels near the Wilmington Air Park in Wilmington, Ohio. The analogs detected were 4-methyl-1H-benzotriazole and 5-methyl-1H-benzotriazole: known together as tolytriazole (TTZ). BTZ and TTZ are emerging environmental contaminants of concern that are often found in aircraft de-icing solutions, anti-icing solutions and detergents. The Wilmington Air Park has two facilities used to pre-treat runoff water before its subsequent release into surrounding streams. Three sites were chosen: Lytle Creek, Indian Run, and Cowan Creek. For the 2019 and 2019/2020 investigative projects, Cowan Creek was designated the control site. Eight sample days were completed from November 2019 to March 2020. The method used in this 2019/2020 sample season utilized the solid-phase extraction (SPE) method and maintained analysis by liquid chromatography-mass spectroscopy (LC-MS). Ultimately, BTZ was below limits of detection at either the Cowan Creek or Indian Run site. BTZ was detected on seven of the eight sample days at Lytle Creek. TTZ was detected all sample days at both the Lytle Creek and Indian Run sites. Recovery corrected BTZ concentrations (internal standard was 5,6-dimethylbenzotriazole) ranged from 0.148 to 3.47 µg/L at the Lytle Creek site. Recovery corrected TTZ concentration ranges were 0.725-12.0 µg/L and 0.214-5.66 µg/L for Lytle Creek and Indian Run, respectively. This would seem to indicate that the treatment facilities are not 100% effective, and that air traffic may have increased. The sample day with the highest TTZ concentrations was the

coldest. Correspondingly, the lowest concentrations were days with the highest ambient temperature. Observed BTZ concentrations were within reported literature ranges, while TTZ concentrations were significantly higher than others reported. The concentrations detected would be considered below levels of acute toxicity to aquatic species, chronic toxic effects cannot be ruled out.

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Acknowledgements

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1. Introduction

1.1 Contaminants of Emerging Concern: Benzotriazole Compounds

In recent years, concern over environmental deterioration has burgeoned. Increasing concern has led to more widespread and detailed monitoring of the impact left by human activities, giving rise to a category of compounds called contaminants of emerging concern (CECs). CECs are compounds that are becoming increasingly prevalent and detectable at low concentration levels.[1] CECs can be sourced to many human activities such as industry, transportation, pharmaceuticals and person care products. The presence of CECs in the environment demands attention due to their unknown potential to negatively impact ecological or human health.[2] Benzotriazoles (BTZ) and analog compounds have joined the infamous ever-expanding list of CECs.

Benzotriazoles are a classification of bicyclic compounds comprised of a benzene ring with a 1, 2 attachment to three nitrogens creating a second ring. Benzotriazole (BTZ) has many derivatives, but two commonly derived isomers are 4-methyl-benzotriazole (4-MBTZ) and 5-methyl-benzotriazole (5-MBTZ). Tolytriazole (TTZ) refers to a mixture that is primarily comprised of just 4- and 5-methyl-benzotriazole but can occasionally contain trace amounts of the 6- and 7-methylated isomers. Benzotriazole can be modified to have a wide range of substituents beyond the addition of a methyl- group as found in 4- and 5-methyl-benzotriazole. Substituent groups that are commonly added include halogens, acyl, and phenolic groups; additionally, these substitutions can occur on any one of the three nitrogens or on the available carbons. Figure 1 shows the structures of benzotriazole (*a.*), 4-methyl-benzotriazole (*b.*) and 5-methyl-benzotriazole (*c.*).

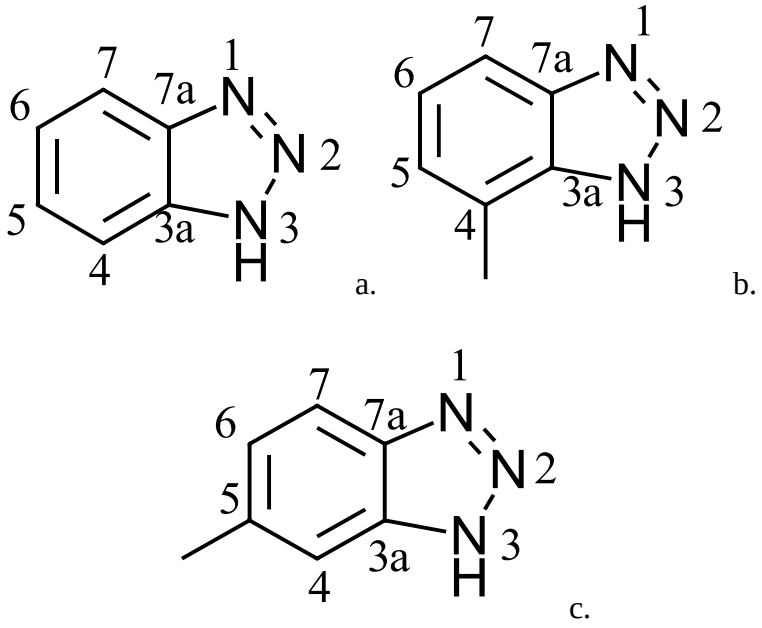

Figure 1 BTZ and TTZ structures: a. benzotriazole, b. 4-methyl-benzotriazole and c. 5-methyl-benzotriazole.

BTZ and TTZ have very similar properties, some of which aid in making them an environmental nuisance. Their properties are tabulated in Table 1 shown below.

Table 1 Benzotriazole and tolytriazole properties.[3],[4],[5]

Feature	Benzotriazole	Tolytriazole
Formula	$C_6H_5N_3$	$C_7H_7N_3$
Molecular Weight (g/mol)	119.12	133.17
Melting Point (°C)	98.5-100	76-87
Physical Description	White to light beige powder or flakes without odor	Light brown flakes with odor
log K_{oc}	1.02	1.68
pK_a	8.2	8.9
pK_b	5.8	5.1
Water Solubility (g/l)	28	7
Other solubilities	Alcohol, benzene, toluene, chloroform, DMF	Ethanol, Acetone, methanol, isopropanol, ethylene glycol, toluene
Density (g/cm^3)	1.36	1.24
UV Absorbance (nm)	286	396

BTZ as an undissolved solid is a white to light beige powder or flake without a perceptible odor. TTZ is usually characterized by light brown flakes accompanied by an odor. Both compounds are nonvolatile and soluble in many organic solvents and are also soluble in water.[3] The water solubilities of BTZ and TTZ are 28 g/L and 7 g/L, respectively. These moderate water solubilities make TTZ and BTZ decently mobile in the hydrologic cycle and further complicates their use and environmental containment. Additionally, these compounds both have reported $\log K_{oc}$ and $\log K_{ow}$ (BTZ: 1.02/1.23 and TTZ: 1.68/1.89) that would increase their proclivity to remain aqueous rather than sorbing into the surrounding soil or sediment in a natural aquatic environment; however, some sorption would occur.[5] $\text{Log}K_{oc}$ and $\log K_{ow}$ describe related compound features: a compounds sorption preference to soil or sediments and a compounds tendency to bioaccumulate. Compounds with a relatively high $\log K_{oc}$ and $\log K_{ow}$ are more inclined to adhere and remain in soil. Combined with these features and the fact that BTZ and TTZ are resistant to bio- and UV-degradation, it would seem that once introduced into an aquatic environment, they often have few pathways for being removed.[4],[5]

The limited modes of removal for benzotriazoles are of great concern due to their widespread variety of uses and high production volume, being estimated at least 9000 tonnes per year worldwide.[5] In fact, a single chemical manufacturer reports that they produce at least 4990 tonnes and 4000 tonnes of BTZ and TTZ respectively: which is primarily exported to Europe, the United States and Southeast Asia. BTZ and TTZ are frequently used as anti-corrosives and in de-icers. Commercially, BTZ and TTZ can be found in aircraft de-icers.[5] Other benzotriazoles are used in a myriad of products including fungicides, UV-stabilizers, photographic antifogging agents, dish washing detergents, and dyes.[3] This manufacturer also states that they have a dual wastewater treatment system, that is comprised of a wastewater treatment plant followed by a

constructed wetland.[6] This double treatment system would appear to be a more optimal arrangement, when considering a reported 20-70% removal efficiency for benzotriazole using a conventional wastewater treatment process and 89-93% using constructed wetlands. Degradation in constructed wetlands is proposed to be attributable to biodegradation, photodegradation and plant uptake [5],[7]

1.2 Previous Findings

1.2.1 Plant and Animal Toxicities

As benzotriazole production and use has grown, understanding their impact upon the environment and living organisms has increased. These interests have been explored in multiple studies: with the organisms most studied to determine benzotriazole health effects, or lack thereof, being plants. Other studies have been conducted to observe the potential effects on assorted small animals but seemingly to a lesser extent.

A study conducted by Wu *et al.* involved five different kinds of plants and a fungus. The plants and fungus used in the study were: pumpkins, cottonwood and corkscrew willow cuttings, horseradish, alfalfa and the fungus *Phanerochaete chrysosporium.* The plants were grown to a certain low level of maturity and then exposed to various levels of benzotriazole and methylbenzotriazole. Most plants involved in the study died shortly after exposure, ranging from two days to four weeks, except for horseradish and the fungus. Pumpkin plants exposed to a benzotriazole concentration of 500 ppm to soil died within 2 days, while pumpkin plants exposed to a concentration of 50 ppm in water died within 10 days. Alfalfa plants exposed to 500 ppm to soil died within 2 to 3 weeks. Besides plant death, exposure resulted in inhibition of plant growth. Both the horseradish and the fungus actually seemed to decrease the concentration of benzotriazole present in their growth medium after exposure. The study also notes that it has

been previously seen that benzotriazoles have morphological effects, such as stem thickening, inhibition of internodal elongation and suppression of root lengthening. These occurrences may result from the structural similarities between benzotriazoles and plant hormones such as auxin.[8] Auxin is a plant hormone that regulates cell elongation, among other growth-related functions.[9]

A 2015 study by LeFevre *et al.* studied the effects of benzotriazole on *Arabidopsis thaliana* plants grown hydroponically and exposed to low levels of benzotriazole. Plants were exposed to 3 µg/L, upon which some plants were taken after exposure and other plants were taken daily over three days after exposure. The low concentration level used was considered environmentally relevant. Harvesting of plants continued over an eight-day period. Analyte was extracted using an SPE process and then analyzed using LC-MS and LC-QTOF-MS. The study concluded that BTZ was rapidly absorbed by the hydroponically grown *Arabidopsis* plants. No leaching of the BTZ compound was observed from the plants during depuration testing: indicating that the compound may be irreversibly absorbed by the plant. Additionally, evidence was found supporting the idea of benzotriazole transformation into benzotriazole-based metabolites closely representing naturally occurring plant hormones. Benzotriazole metabolites observed closely resembled tryptophan and auxin. Tryptophan in plants is used as a synthesis precursor for a number of other vital processes. Benzotriazole based compounds similar to other naturally produced compounds could potentially have detrimental health effects on those in the next step of the food chain, as tryptophan is an essential amino acid for animals: used to synthesize proteins and other biological activities.[10]

In a 2017 conducted by LeFevre *et al.* further evidence indicating the transformation of BTZ into plant metabolites was observed using strawberry and lettuce plants. Plants used in the

study were grown in soil and water with highly recycled wastewater spiked with environmentally relevant levels of BTZ (0.027-0.279 µg/L) multiple times a week. Samples were analyzed by LC-ESI-MS/MS. BTZ was detected in all plant tissues and even found in control samples which ultimately resulted in the discovery of trace levels of BTZ in the areas tap water. Glycosylated-BTZ and BTZ-acetylalanine was found in strawberry plant tissue samples; however, they were not found in the fruit of the plant and metabolites were not found in lettuce plants.[11]

BTZ animal studies are scarcer than plant related BTZ studies and mostly involve small non-mammal aquatic species. The main source located for animal toxicities involving mammals was a Danish health evaluation regarding BTZ and TTZ published in 2013. [3] The study presented single and repeated dose toxicities through various modes of exposure for a variety of small mammals including rabbits, rats, mice and guinea pigs. Modes of exposure included inhalation, oral intake, and dermal contact. Data conglomerated in the study regarding observable effects and lethal concentrations required to kill 50% of the tested population (LC_{50}) are show in Table 2.

Table 2 Observable health effects and LC_{50} for TTZ and BTZ in small mammals.[3]

Subject	Mode of Exposure	Dose	Observations	LC_{50}
Mice	Inhalation	Single	Respiratory irritation, depressed respiration	N/A
	Oral Intake	Single/Repeated	Weight loss, bone, kidney, lymphatic damage (Repeated/low dose) Potential carcinogen	Single BTZ: 615-831 mg/kg Single TTZ: 800 mg/kg
Rat	Inhalation	Single	Liver/Kidney damage. Respiratory Irritation	BTZ: 1910 mg/m^3 TTZ: >1730 mg/m^3
	Oral Intake	Single/Repeated	Death within 2 days/ weight loss, lethargy, reproductive damage, neurotoxcity. Liver, lung, CNS, and digestive damage	Single, BTZ:500-965 mg/kg
Guinea Pig	Oral Intake	Single	Potential acute CNS toxicity	BTZ: 500 mg/kg
	Intra-dermal injection	Repeated	No strong observations	N/A
Rabbit	Dermal Contact	Single	No strong observations	TTZ: >>2000 mg/kg

For inhalation studies, benzotriazole and tolytriazole were aerosolized and introduced to a group of rats. Inhalation resulted in test subject death over a wide exposure range between 780 to 2790 mg/m^3 with an LC_{50} of 1910 mg/m^3. Aerosolized tolytriazole exposure for one hour on rats resulted in liver and kidney damage in addition to unsurprising respiratory irritation. The proposed tolytriazole LC_{50} was greater than 1730 mg/m^3. Rats that were used to test the effects of benzotriazole consumption were force fed using a gavage. The LC_{50} for rats ingesting benzotriazole is in the range of 500 to 965 mg/kg. Death followed within 2 days of fatal dose administration. At lower levels and over a longer exposure time, weight loss, lethargy and acute neuro toxicity were reported, in addition to damage to the reproductive system. Rats given

tolytriazole between 1 to 100 mg/kg per day for 2 weeks showed no signs of toxicity; however, rats given 500 mg/kg per day or more showed signs of liver, lung, central nervous system and stomach effects. Rabbits used for dermal testing fared better than the rats used in previously mentioned studies. Groups of rabbits were shaved and had one application of benzotriazole applied to their skin for 24 hours. No rabbits died from this dermal test. A reported LC_{50} value for tolytriazole was indicated to be well over 2000 mg/kg, along with no strong observations.

Smaller aquatic non-mammal studies included various fish. Short term studies have found that bluegills and minnow have a benzotriazole tolerance level of up to 27.5 mg/L and 25 mg/L after 96 hours of exposure. Trout have been found to have a lower tolerance level: 15 mg/L for 48 hours and 12 mg/L after 96 hours of exposure. A higher fish mortality rate is observed after 96 hours than after 48 hours. This could suggest that benzotriazole becomes stored in tissues and accumulates eventually leading to death.[4] Needless to say, the lack of available studies (and more recent studies) into effects upon living organisms regarding BTZ and TTZ along with the increasing prevalence of detection in the environment lends to the urgency for more investigations.

1.2.2 Worldwide Environmental Studies

Studies investigating the environmental fate and appearance of BTZ and TTZ have primarily been conducted in Europe, Asia and North America but a small number of studies have also been completed in Australia. These studies inspected varying areas, such sediments and wastewater, and had wide ranging results. The results from studies conducted in eight different countries can be seen below in Table 3.

Table 3 Studies with location, analyte concentrations and instrumentation worldwide.

Study	Location	Instrumentation	BTZ	TTZ
Alotaibi et al. (2015)[12]	Australia	LC-MS	Surface water: 0.011-0.079 µg/L	Surface water,5-MBTZ: 0.002-0.046 µg/L
Parajulee et al. (2017)[13]	Canada	LC-MS/MS	Surface Water: 0.00091–2.390 µg/L	Surface water,4-MBTZ: 0.00044–1.990 µg/L Surface water,5-MBTZ: Non-Detect–0.448 µg/L
Kiss *et al.* (2009)[14]	Germany	GC-MS	Surface Water: 0.038 – 1.474 µg/L	Surface Water, 4-MBTZ: 0.025 – 0.952 µg/L Surface Water, 5-MBTZ: 0.025 – 0.281 µg/L
Asimakopoulos *et al.*(2009)[15]	Greece	LC-MS/MS	Wastewater sludge: 0.081-0.084 µg/g	Wastewater sludge: 0.116 µg/g
Karthikraj *et al.* (2017)[16]	India	LC-ESI(+)MS/MS	Average Wastewater Influent: 0.0787 µg/L	Average Wastewater Influent: 0.200 µg/L
van Leerdam *et al.* (2009)[17]	Netherlands	LTQ-FT-Orbitrap-MS	Max Wastewater Effluent: 8 µg/L	Max Wastewater Effluent: 3 µg/L
Giger *et al.*(2006)[4]	Switzerland	LC-MS/MS	Max Surface Water: 6.4 µg/L	Max Surface Water: 0.47 µg/L
Janna *et al.* (2011)[18]	UK	ESI(+)-triple quadrupole MS	Surface Water: 0.013–1.960 µg/L	Surface Water: 0.020–3.970 µg/L
Alvey *et al.* (2016)[19]	USA	LC-MS/MS	Snow Melt, Average: 0.08 µg/L	Snow Melt, Average: 0.59 µg/L

As one can from the above table, most methods of analysis looking into the appearance of BTZ and TTZ used more complicated analytic instrumentation than LC-MS. Multiple studies used tandem mass spectrometry accompanied by other instrumentation systems such as gas chromatography (GC) and linear trap quadrupole (LTQ) Orbitrap. Studies in North America found concentrations of BTZ at an average of 0.08 µg/L in snow melt and 0.91 ng/L minimum in surface water. North American detected maximum concentrations in surface water were found to be 2.390 µg/L by Parajulee *et al.* in the Canadian-based study. This study also found 4-MBTZ

and 5-MBTZ below 2 µg/L and 0.5 µg/L respectively. A German study conducted by Kiss *et al.* found BTZ concentrations ranging from 0.039-1.474 µg/L, 4-MBTZ in a range of 0.025 – 0.952 µg/L and 5-MBTZ in a range of 0.025–0.281 µg/L in surface waters. A Swiss study by Giger *et al.* found BTZ and TTZ levels below 10 µg/L in surface waters. A study completed by Alotaibi *et al.* investigating the appearance of BTZ and 5-MBTZ in Australian surface waters found concentrations below 1 µg/L. Askimakopoulos *et al.* observed the appearance of BTZ and TTZ in Greek wastewater treatment plants and found concentrations of BTZ and TTZ below 1µg/g in wastewater sludge. Two similar studies looking into the occurrence at wastewater treatment plants in India and the Netherlands were conducted as well. Karthikrai *et al.* found average BTZ and TTZ concentrations of 0.0787 ng/L and 0.200 ng/L in Indian wastewater influent. The Netherlands study by van Leerdam *et al.* found max wastewater effluent BTZ and TTZ concentrations of 8 µg/L and 3 µg/L respectively: these are the highest levels detected in the studies included.

1.2.3 Previous sampling results

The 2019 sampling season performed by Jessica Weise started February 1st, 2019 and ended on February 28th, 2019. Sampling was completed approximately once a week at three sample sites and produced five sample days. Sample sites included Cowan Creek (CCJKR), Indian Run (IRJKR) and Lytle Creek (LCFR). Cowan Creek was the control site used for the 2019 and 2020 sampling season. No analytes of interest were found at the Cowan Creek sample site, indicating that the source of BTZ/TTZ was likely from airpark run off and not another source. BTZ was not found at the Indian Run site; however, the analyte was found at the Lytle Creek site but not in quantifiable levels. The Lytle Creek and Indian Run samples contained detectable levels of TTZ on all sample days during the 2019 season. TTZ levels at the Indian Run sample site were lower

than at Lytle Creek, ranging from 0.111-1.248 µg/L versus 0.822-3.435 µg/L. It was concluded that these low levels would not have been picked up by air park assessments done monthly. Assessment parameters monitored include chemical oxygen demand (COD), dissolved oxygen (DO), ammonia, total suspended solids, rate of flow and dissolved solids. COD and DO restriction limits are set in the range of mg/L in post treatment runoff. The 2019 sample season also observed a trend connecting levels of TTZ to weather conditions: lower temperatures and more precipitation led to higher concentrations.[20]

1.3 Approach used in this study

The 2019/2020 approach to this project shares the same main goal as the 2019 investigation: determine the concentration of BTZ and TTZ in select surface waters surrounding the Wilmington Airpark. However, the 2019 investigation was nuanced at determining baseline levels of the analytes, BTZ and TTZ, prior to expected air park operations and traffic increasing. After that a major commercial online retailer began leasing at the Wilmington Air Park facility in June 2019[21], adding eight additional flights per day for an average of 14 flights per day as of September 2019.[22] Since then, the airpark has been ranked 33rd out of 780 ports in the nation in terms of pounds of freight shipped in a year (late 2019 to late 2020) and as of December 2020 the air park was first in terms of cargo volume for a one year span finishing in September 2020. These rankings are at least partially attributable to the COVID-19 pandemic and an increased trend in online shopping. [23],[24] The 2019/2020 approached was more specifically interested in analyte concentrations in relation to the previous sampling season's findings and better isolate the analytes through additional method development. It was also expected that the contaminant levels were going to be higher than the 2019 sample season findings resulting from the rise in air traffic out of this port.

To attain these goals, three sample sites were monitored and sampled over a period of time extending from November 12th, 2019 until March 10th, 2020. A total of eight sample days were completed according to the SOP, shown in Appendix A. Sample sites included two of the exact same sites used in 2019: Lytle Creek (LCFR) and Cowan Creek (CCJKR). However, the corresponding 2019 Indian Run site could not be sampled and a different, but comparable, site had to be chosen. Regrettably, there was a second relocation of the Indian Run site. All three sites are within a 500-meter stretch of each other on the left bank of Indian Run going downstream. One can walk from the first 2019/2020 Indian Run site to the second within 5 minutes. Two replicate samples, split further into three replicates each, were collected from all sites on each sample day. Samples were then frozen in a freezer at approximately -20℃, after which they were processed through a solid-phase extraction (SPE) and analyzed using liquid chromatography-mass spectrometry (LC-MS).

2. Experimental

2.1 Sampling Process

*2.1.1 **Sampling Materials***

The following list describes the materials necessary to prepare for the sampling process and for the sampling procedure itself.

- YSI Multimeter Pro Plus
- YSI Calibration Standards
  - YSI 3161 Conductivity Calibrator Solution (1000 µS/cm ± 0.50% at 25°C)
  - YSI 5580 Confidence Solution
  - YSI 3821 Buffer Solution pH 4.00±0.01 at 25°C
  - YSI 3822 Buffer Solution pH 7.00±0.01 at 25°C
  - YSI 3823 Buffer Solution pH 10.00±0.01 at 25°C
  - YSI 3841 1mg/L NH_4^+ -N Standard
  - YSI 3843 100mg/L NH_4^+-N Standard
- Water (CAS #7732-18-5, ASTM Type I Water, 17.5-MΩ resistance)
- Gloves
- 500-mL amber glass bottles with Teflon® lids
- Cooler and icepacks

*2.1.2 **Sampling Procedure***

Sampling procedures were similar to previous sample methods utilized in the early 2019 investigation [22] but did have a few augmentations. The augmented SOP can be found in Appendix A. The 2019/2020 investigation sample collection began November 13, 2019 and ended March 10, 2020. Eight sample days were collected when weather and circumstances permitted. The day prior to each sample day, the YSI Multimeter ProPlus Instrument was calibrated following SOP 13.0 with corresponding buffers and standards (Appendix A). Two

500-mL glass amber bottles with Teflon caps were thoroughly rinsed with reverse-osmosis purified tap (RO) water and ASTM Type-I water for each sample site, totally six glass amber bottles per sample day, the day prior to sampling. Bottles were then left to dry overnight. As previously described, three sites were sampled. However, one sample day (12042019) was collected by Travis Luncan. For this sample day only one 500-mL amber bottle sample was collected from each site. Additionally, a different water quality probe was used.

Cowan Creek and Lytle Creek sites were the same locations as previously described in the 2019 investigation.[22] However, the Indian Run site had to be relocated twice from the previous investigations site. The first relocation, Indian Run Site 1, was spurred by the discovery that the small access bridge crossing the run had been destroyed. The second relocation, Indian Run Site 2, was located near the airpark but not on airpark property. Indian Run Site 1 was sampled from November 12, 2019 until January 23, 2020: after which, Indian Run Site 2 was used. A satellite view of these locations is shown below in Figure 2. Yellow arrows show the direction of flow. Water flows from the Indian Run water treatment facility follows a path next to farm fields and towards a confluence with Cowan Creek. The water treatment facility near the Lytle Creek site releases run off that takes a path over a small amount of land, then under a road, and through a small area of trees and underbrush, after which the Lytle Creek sample site is located. Sample sites, water treatment beds and facilities are indicated. An additional satellite view of the air park and sample sites can be located in Appendix A.

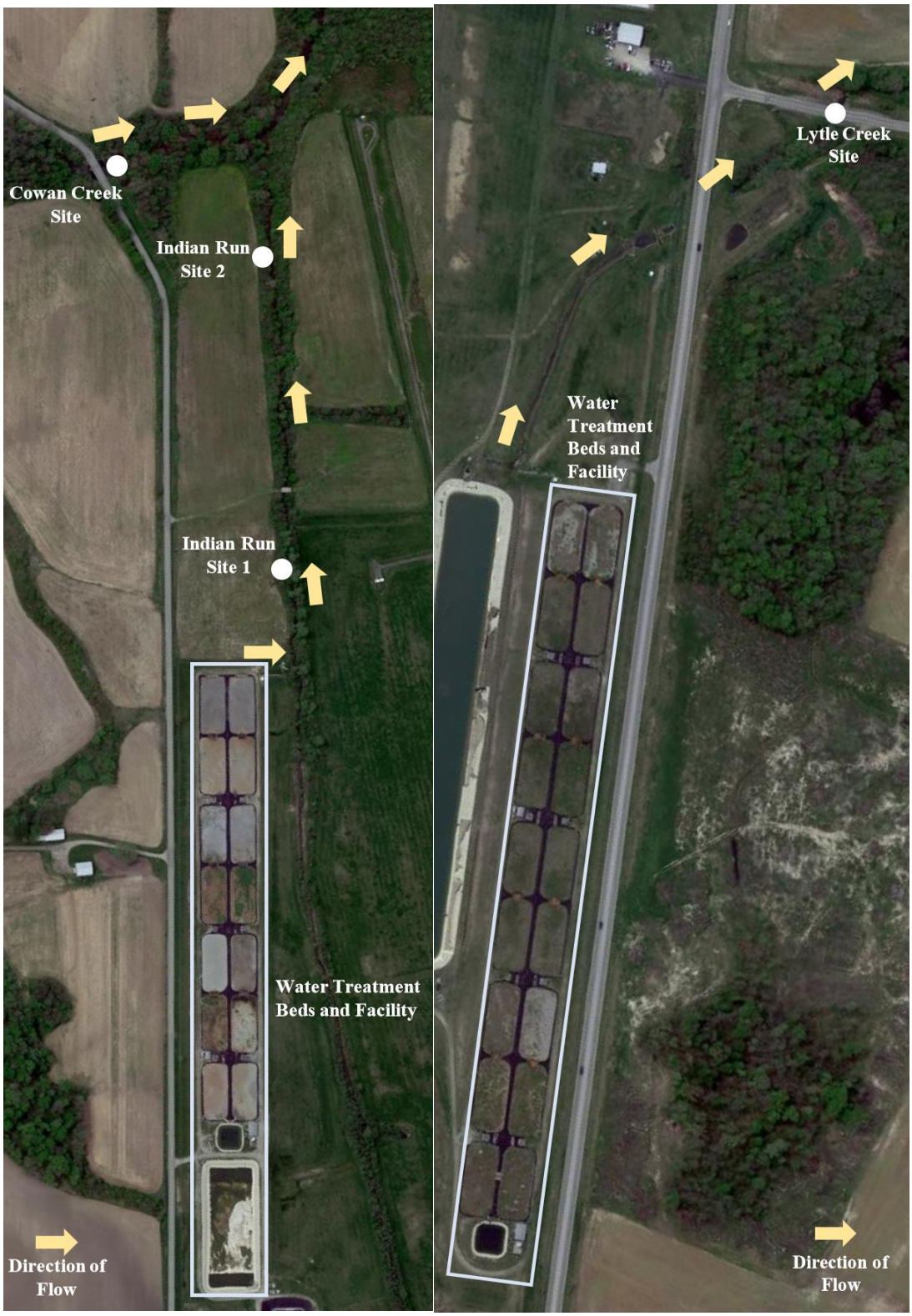

Figure 2 Satellite view of sample sites with direction of water flow, water treatment beds, and facilities displayed.[25]

Below Table 4 displays the geographical coordinates for the three sample sites and includes the coordinates for the Indian Run relocations.

Table 4 Sample site geographical coordinates

Sample Site Name	Coordinates
Cowan Creek	39.407615, -83.798064
Indian Run Site 1	39.411386, -83.795392
Indian Run Site 2	39.408914, -83.799194
Lytle Creek	39.437051, -83.797386

Sample code IDs for Cowan Creek, Indian Run and Lytle Creek were CCJKR, IRJKR and LCFR respectively. Sample code IDs also included date, bottle replicate and eventually sample replicate letter. Sample replicate letters, A through C, were added during processing to indicate 100-mL replicates. An example is shown below.

Date-Site-Bottle Replicate Sample-Replicate Letter

12042019-CCJKR-R1-A

All sample days were conducted, and all sample sites visited, with the accompaniment of Travis Luncan (except for 12042019, which Mr. Luncan collected by himself), who is currently the Source Water Protection Coordinator for the City of Wilmington, Ohio. Mr. Luncan holds a zoology degree from Miami University and a chemistry degree from the University of Cincinnati. Mr. Luncan has immense experience with water quality assessment and analysis: in addition to being familiar with the flora, fauna and geography of the area surrounding the airpark.

The Cowan Creek sample site can be found on the downstream side of the Jenkins Road bridge. A downstream view of the Cowan Creek sample site can be seen below in Figure 3 from February 25, 2020.

Figure 3 Downstream view of Cowan Creek (2/25/20), high water level

Indian Run Site 1 was approximately 0.3 miles down the road towards Old State Route 73. Indian Run Site 2 was relatively close to both the Indian Run Site 1 and Cowan Creek sites. The second Indian Run site could be accessed quickly starting from the Cowan Creek site. A quick diagonal jaunt through a very small, wooded area and a field (approximately 0.1 miles) led to the sample site. Both Indian Run Sites required a small down climb to access the streams or the use of a bucket attached to a rope. Figure 4 displays the upstream view of both Indian Run sites.

Figure 4 Upstream view a. Indian Run site 1 (1/14/20) b. Indian Run site 2 (3/10/20), featuring Travis Luncan.

The Lytle Creek site is located less than 0.1 miles from the intersection if Davids Drive and Fife Avenue. The site can be located approximately 4.5 miles from the Cowan Creek site by road. Figure 5 shows the upstream and downstream view of the Lytle Creek site on March 10, 2020.

Figure 5 Lytle Creek site a. upstream, sample side b. downstream, non-sample side (3/10/20).

The creek is traversed by Fife Avenue and routed through a large metal drainage pipe under the road. Samples taken from the Lytle Creek site were always procured from the upstream side (*a.*) of the drainage pipe. The downstream side (*b.*) was not sampled.

The Two 500-mLTeflon ® lidded amber bottles were filled with 300-400 mL of water sample from each site. This was done by directly lowering the bottle into the water and collecting near the surface: after rinsing the bottle three times with sample site water. If necessary, a bucket (constructed of a cut open sturdy jug and a rope) could be lowered into the water. The bucket was also rinsed with sample site water prior to using it to collect sample. Bottles were then placed into a small cooler containing plastic packing material and ice blocks for transport.

Water quality data was then collected using the previously calibrated YSI Meter. Parameters monitored throughout sampling included: ambient and water temperature (°C); specific conductance and conductivity (µS/cm); percentage and mg/L dissolved oxygen; ammonia (NH_3) and ammonium (NH_4^+); pH and pressure (mmHg). Samples were transported to Wright State University Dayton Campus and stored in a freezer set to -20°C until sample processing. Standard Operating Procedure for YSI data collection and water sample collection can be found in the Appendix A. The data form used in recording water quality data can be found in the Appendix A as well.

2.2 Solid-Phase Extraction Process

2.2.1 Solid-Phase Extraction Materials

The following materials were used in the solid-phase extraction (SPE) treatment of collected select surface water samples from the area near Wilmington Air Park.

- OASIS HLB Cartridges (Waters Inc. 500-milligrams, 6 mL)
- 0.7-µm glass fiber filters (Whatman, GF/F 47 millimeter)
- 15-mL graduated centrifuge tubes (Kimax, Kimble-Chase Glassware)
- Nitrogen, gas (CAS #7727-37-9, Airgas Operations, Ultra High Purity 5.0 Grade)
- Water (CAS #7732-18-5, ASTM Type I Water, 17.5 MΩ resistance)
- Hydrochloric acid (12 M HCl, CAS #7647-01-0)
- Glass Pasteur pipettes

2.2.2 Chosen Solid-Phase Extraction Method

The solid phase extraction (SPE) method initiated by pulling the samples out of the freezer (approximately -20℃) and thawing them out in a refrigerator (approximately 4 ℃) for at least 24 hours. Thawing was done in the refrigerator, as opposed to at room temperature in an attempt to avoid breaking the amber bottles which contained the samples. The liquid samples were then filtered through 0.7-µm Whatman glass fiber filters using a funnel and flask setup under vacuum. After filtration, the sample were aliquoted into 100-mL replicates. Each replicate was acidified using five drops of concentrated hydrochloric acid (HCl) to a pH of approximately 2.5-3 and spiked with 50 ng (10 µL of 5.0 ppm standard) 5,6-dimethyl-benzotriazole which serves as the internal surrogate standard.

The replicates were then filtered through pre-conditioned OASIS HLB Cartridges. OASIS HLB Cartridges were conditioned with three treatments of 2 mL methanol and three treatments of 2 mL Milli-Q water. After filtering the samples through the cartridges, they were dried under a gentle vacuum (approximately -15 psi) for approximately 2.5 hours. The sample analyte was then eluted into centrifuge tubes using 5 mL of 3% methanol in dichloromethane (DCM) under gentle vacuum. Upon completing elution, the sample replicates were evaporated to dryness using a gentle stream of nitrogen gas. Initially, evaporating one sample took approximately fifteen minutes. Considering the number of samples procured in this sample season (126 individual replicates), this would be a rather arduous process and would not have been a judicious use of time. In order to minimize the evaporation time, while maintaining sample integrity, a water bath was set up to keep samples aid in evaporation (exceeding no more than 35 ℃). While samples were not actively being dried under nitrogen, they were held in the water bath. This method decrease sample drying time by five minutes per sample. The dried analyte was then reconstituted in 1 mL of methanol and stored in a freezer (approximately -20 ℃) overnight. The samples were then transferred to autosampler vials the next day: after which, the samples were stored in the freezer until LC-MS analysis.

2.2.3 **Solid-Phase Extraction Method Development and Validation**

SPE method confirmation was done using two mock sample sets. Both the first and second mock sample set was manufactured using tap water, 10.02 µg/L BTZ and TTZ standards. A 1 L mock tap water sample was created using 0.500 mL of the 10.02 µg/L BTZ standard and 250 µL of each the 4-methyl-benzotriazole and 5-methyl-benzotriazole. The two isomer concentrations were summed as the instrument is incapable of separating them. This should have given a concentration of 5.01 µg/L BTZ and 5.01 µg/L TTZ. A detailed standard preparation

procedure can be found in Appendix B. All prepared standards ranged from 10.02 µg/L to 100.2 µg/L: this ranged was used for all calibration curves. All 1 L of the mock sample was run through the SPE process as described in section 2.2.2. Replicates for the first mock sample set were indicated as 01042021 R1 through R5: An unfortunate mishap happened when some amount of R2 was poured into the R1 OASIS HLB Cartridge during cartridge filtration. There was additional sample loss during transfer to SPE cartridges. These errors can be seen reflected in non-optimal percent recoveries shown in Table 5. A general unfamiliarity with the process most likely contributed considerably as well.

Table 5 Tap water mock sample set 1, replicate percent recoveries.

Replicate Number	.6-Dimethyl-Benzotriazole Recovery (%)	Benzotriazole Recovery (%)	Tolytriazole Recovery (%)
01042021-R1	71.6	15.7	57.1
01042021-R2	37.9	1.99	19.3
01042021-R3	35.5	0.567	3.42
01042021-R4	34.1	0.829	6.05
01042021-R5	43.0	3.34	25.1
Average	37.5 ± 4.75	4.59 ±6.37	22.2 ±21.5

Considering the near abysmal percent recoveries from the first mock sample set, the second run was deemed necessary using the remaining 500 mL of mock sample. The second mock sample set was indicated as 01152021 R1 through R5. Very minor changes were made to this second SPE run. This included an additional 2 drops of acid being added to the 100 mL sample replicates and pipettes were used to transfer the sample replicates to respective cartridges. These minor changes resulted in a better percent recovery and can be seen in Table 6.

Table 6 Tap water mock sample set 2, replicate percent recoveries.

Replicate Number	5.6-Dimethyl-Benzotriazole Recovery (%)	Benzotriazole Recovery (%)	Tolytriazole Recovery (%)
01152021-R1	66.9	76.9	74.5
01152021-R2	60.9	67.6	64.1
01152021-R3	83.6	81.2	78.0
01152021-R4	67.8	72.0	68.6
01152021-R5	69.6	75.3	69.8
Average	69.8 ± 5.4	74.6 ± 5.1	71.0 ± 5.4

The second mock sample set process resulted with better percent recoveries. The internal standard (5,6-DMBTZ) had a percent recovery of 69.8% ± 5.4. BTZ and TTZ percent recovery could be calculated for the mock sample sets since a controlled amount was added in lab. The percent recovery for BTZ and TTZ percent recoveries were 74.6% ± 5.1 and 71.0% ± 5.4, respectively. The average percent recovery for this mock sample set was 71.7% ± 2.3. These percent recoveries were deemed high enough and reproducible enough to proceed with actual sample processing.

2.3 Liquid Chromatography-Mass Spectroscopy Analysis

2.3.1 Liquid Chromatography-Mass Spectroscopy (LC-MS) Materials

The list below contains materials utilized in LC-MS analysis throughout method development and sample treatment. Along with the listed names of materials, the CAS number, producer and purity level are detailed.

- 1H-benzotriazole (CAS #95 14-7, Sigma-Aldrich, ≥98.0% purity)
- 4-methyl-1H-benzotriazole (CAS #29878-31-7 Sigma-Aldrich, ≥90.0% purity)
- 5-methyl-1H-benzotriazole (CAS #136-85-6 Sigma-Aldrich, ≥98.0% purity)
- 5,6-dimethyl-1H-benzotriazole (CAS #4184-79-6, Chem Bridge, 100% purity)

- Methanol (CAS #67-56-1, Fischer Scientific, HPLC-Grade, 99.9% purity)
- Water (CAS #7732-18-5, ASTM Type I Water, 17.5-MΩ resistance)
- Dichloromethane (DCM, CAS #75-09-2, Fischer Scientific, 99.9% purity)
- Formic Acid (CAS #64-18-6, Fischer Scientific, LC/MS-Grade, ≥99.0% purity)
- Nitrogen gas (CAS #7727-37-9, Airgas Operations, Ultra High Purity 5.0 -Grade)

2.3.2 **LC-MS Method**

The LC-MS method that was developed and chosen for sample analysis is similar to the previous iteration of this projects method. However, a few changes were made to optimize the method according to the aims of this seasons project continuation. The instrument used was an Agilent Technologies 1220 Infinity LC with a variable wavelength detector paired with a quadrupole mass spectrometer using electrospray ionization (ESI). The instrument also included the use of a C18 (1.8-µm I.D. 2.1 x 100-mm) column and autosampler to separate the BTZ and TTZ analytes of interest. The injection volume for all samples and standards was 2µL at a flow rate of 0.120 mL/min. The eluent ratio used was 45:55 water to methanol both containing 0.1% formic acid. Due to the slower flow rate, the total scan time was extended to seven minutes. The column temperature was kept at 25℃ with a ±0.8℃ allowance. Column pressure limits were restricted between 50.0 bar lower threshold and 360.0 higher threshold. The variable wavelength detector (VWD) was set to scan between 190 nm and 400 nm with a signal of 273 nm.

The mass spectrometer was used under positive ionization (PION) and single-ion monitoring (SIM) mode. Molecular ions monitored for BTZ, TTZ and 5,6-DMBTZ (surrogate standard) were at *m/z* 120, *m/z* 134 and *m/z* 148, respectively. These values were chosen to keep consistency between these years work and previously done. Examples chromatograms for the standards can be found in Appendix B accompanied by respective mass spectrums. TTZ isomers

(4-methyl-benzotriazole and 5-methyl-benzotriazole) were not able to be separated in this analysis; however, chromatograms and MS spectra are shown in the appendix where it can be seen that the isomers elute at the same time and can be seen using the same molecular ion. Also observable is a slight difference in peak height. The 4-methyl-benzotriazole isomer appears to result in a higher chromatogram peak than a 5-methyl-benzotriazole sample of equivalent concentration. Peak integration was done manually using OpenLAB CDS Chemstation Software for all chromatograms.

Calibration curves were created using BTZ, TTZ and 5,6-DMBTZ standards for each sample run. Created standard concentrations for all three sets ranged from 10.02 µg/L to 1002 µg/L; however, only 10.02 µg/L to 100.2 µg/L was used to create calibration curves. Using the calibration curves, accounting for the concentration factor (100) and percent recovery the concentration of analyte contained in the samples was determined. The full SOP and calibration curves can be found in Appendix B.

2.3.3 **LC-MS Method Development and Validation**

The LC-MS method development primary focused on flow rate and elution ratio exploration. Previous work utilized a flow rate of 0.140 mL/min; however, due to the desired to achieve greater peak separation two other flow rates of 0.100 mL/min and 0.120 mL/min were initially examined using a 40:60 0.1% formic acid in water (H_2O) and 0.1% formic acid in methanol (MeOH) eluent mix. Minimal peak shift was observed between flow rates; however, peaks did elute quicker using the 0.120 mL/min flow rate. This can be seen in Table 7 below.

Table 7 Flow rate peak elution times

Fow Rate (mL/min)	BTZ Elution (min.)	TTZ Elution (min.)
0.100	4.127-4.129	5.010-5.027
0.120	3.393-3.420	4.040-4.157
0.140[20]	2.921	3.577 (4-MBTZ) 3.519 (5-MBTZ)

The flow rate of 0.120 mL/min was chosen due to quicker peak elution times and in consideration of pressure restriction previously observed in the 2019 investigation. Additionally, the 2019 investigation had found that certain eluent ratios resulted in column pressure limits being exceeded at a flow rate of 0.140 mL/min. Eluent ratios (0.1% formic acid in H_2O: 0.1% formic acid in MeOH) examined in the 2019/2020 investigation using a 0.120 mL/min were: 30:70, 40:60, 45:55, 47:53, 49:51 and 50:50. Table 8 displays BTZ and TTZ peak elution times under the tested condition.

Table 8 Elution times under various eluent ratios at 0.120 mL/min.

Eluent Ratio (0.1% formic acid in H_2O: 0.1% formic acid in MeOH)	**Benzotriazole (BTZ), minutes**	**Tolytriazole (TTZ), minutes**
30:70	3.137	3.200
40:60	3.396	4.056
45:55	3.664	4.706
47:53	3.765	4.932
49:51	3.896	5.401
50:50	4.129	5.923

The eluent ratios of 30:70 and 40:60 resulted in unresolved and merged benzotriazole and tolytriazole peaks. Most pronounced separation of analyte peaks occurred in eluent ratios 47:53, 49:51 and 50:50. However, as the ratio of 0.1% formic acid in H_2O increased the baseline quality degraded and additional elution time was required. Additionally, all three of the aforementioned ratios began to show inklings of tolytriazole isomer peak resolving. The 45:55

(0.1% formic acid in H_2O: 0.1% formic acid in MeOH) resulted in the best combination of baseline quality and peak separation. Given these factors, the 45:55 0.1% formic acid in H_2O: 0.1% formic acid in methanol eluent ratio was chosen for the final method. Using the 45:55 eluent mix with a flow rate of 0.120 mL/minute the analyte peak elution times were determined for this specific method and can be found below in Table 9.

Table 9 Analyte elution times for 45:55 eluent mix at 0.120 mL/minute with standard deviation.

Analyte	**Elution Time** (min.)
Benzotriazole (BTZ)	3.729 ±0.082
Tolytriazole (TTZ)	4.820 ± 0.011
5,6-Dimethyl-Benzotriazole (56-DMBTZ)	6.191 ± 0.012

All injections were 2.00 µL. Chromatograms can be located in Appendix B.

3. Results and Discussion

3.1 Water Quality Measurements and Weather Data

Water quality measurements and data were collected each day at each sample site using the YSI meter. Table 10, below, contains the averages and standard deviations for all water quality parameters monitored throughout the 2019/2020 sampling season.

Table 10 Sample site YSI probe averages with standard deviations

Parameter	LCFR	IRJKR	CCJKR
Ambient Temperature (°C)	1.74±6.99	2.01±6.59	1.87±6.91
Water Temperature (°C)[b]	6.37±2.34	5.49±2.35	4.92±2.77
DO (%)	83.9±13.3[a]	83.7±10.1	88.3±8.84
DO (mg/L)	10.3±1.8[a]	10.4±1.68	11.3±1.39
pH[b]	7.50±0.34	7.40±0.34	7.56±0.29
NH_4^+ (mg/L)	0.60±0.35	0.28±0.11	0.17±0.08
Conductivity(uS/cm)[b]	528±181	423 ± 87	351±51
Specific Conductance (uS/cm)[b]	830±305	679±147	586±102
Pressure (mmHg)	736±5	736 ± 5	736±5

[a] Indicates dissolved oxygen data from 02112020 was omitted due to meter error.
[b] Indicates meter data from 12042019 was available and included.

Not all parameters taken by the YSI meter were available from sample day 12042019; however, water temperature, specific conductance, conductance and pH were available and included. Standard deviations vary greatly due to the span of time data was collected, starting mid-November 2019 and ending early March 2020. High standard deviations may also be attributable to Ohio's proclivity for seemingly unpredictable and fluctuating weather patterns. Most water quality parameters, such as temperature measurements and pressure, did not vary greatly between sites; however, the LCFR sample site appears to have had the highest average conductivity (uS/cm), specific conductance (uS/cm) and NH_4^+ concentrations (mg/L). Higher specific conductance here potentially could indicate that the site experiences greater runoff from the water treatment beds or roadways on this side of the airpark. Salt runoff from efforts to

prevent road icing could greatly contribute to higher conductivities from dissolved salt ions. Table 11 displays water quality parameter data as ranges. Specific values for each parameter can be found in the Appendix A for all seven sampling days.

Table 11 Sample site YSI probe ranges

Parameter	LCFR	IRJKR	CCJKR
Ambient Temperature (°C)	-10-12.2	-8.89-12.2	-10-12.2
Water Temperature (°C)[b]	3.2-10.6	2.4-9.8	0.5-9.6
DO (%)	77.6-95.8[a]	69.7-94.8	73.3-98
DO (mg/L)	7.62-12.8[a]	8.00-12.7	9.16-12.15
pH[b]	6.97-7.85	6.94-7.87	6.92-7.82
NH_4^+ (mg/L)	0.29-1.19	0.19-0.48	0.08-0.28
Conductivity(uS/cm)[b]	232-758	276-565	264-427
Specific Conductance (uS/cm)[b]	321-1245	447-865	422-743
Pressure (mmHg)	726-743	727-743	726-743

[a] Indicates dissolved oxygen data from 02112020 was omitted due to meter error.
[b] Indicates meter data from 12042019 was available and included.

Table 11 indicates that the highest specific conductance (uS/cm) was observed at LCFR, as well as: conductivity (uS/cm) and NH_4^+ (mg/L). The table below contains weather descriptions for three days leading up to and day of sampling. Data and descriptions displayed are a combination of personal observations and data taken from the National Weather Service[26]. "Not enough precipitation to measure" is denoted as NEPTM.

Table 12 Weather observations and data three days prior to sampling and day of.

3 Days Prior	2 Days Prior	1 Day Prior	Day of Sampling	Sample Date
Avg. -6.7 ℃; Almost completely Overcast, Fog; 0.46 cm of precipitation	Avg. -6.7 ℃; Overcast, Wind, Fog and Haze; 0.05 cm of precipitation	Avg. -8.3 ℃; Clear skies, some fog	Cold; slight wind; small amount of snow on ground	11-13-2019
Avg. 2.8 ℃; Clear Sky, Fog and Haze	Avg. 3.3 ℃; Mostly Overcast, Fog and Haze	Avg. 6.1 ℃; Overcast, Fog; NEPTM	Cool; Cloudy; Very Muddy	11-20-2019
Avg. 7.2 ℃; Very overcast, Fog; 0.41 cm precipitation	Avg. 1.7 ℃; Completely Overcast, Fog; 0.15 cm precipitation	Avg. 0 ℃; Extremely overcast, Fog; NEPTM	Mostly clear sky	12-04-2019[a]
Avg. 3.3 ℃; Overcast, Fog, Windy; 1.24 cm of precipitation	Avg. 6.7 ℃; Very Overcast, Fog, Windy; NEPTM	Avg. 6.1 ℃; Partly Overcast	Cold; Very Muddy: Fog	01-14-2020
Avg. -6.7 ℃; Pretty Overcast; NEPTM	Avg. -6.7 ℃; Little Cloud cover	Avg. -2.8 ℃; Clear	Overcast; Mostly Dry; little mud; 0.03 cm of precipitation	01-23-2020
Avg. -1.1 ℃; Completely Overcast, Fog; 0.15 cm of precipitation	Avg. 0 ℃; Very Overcast, Thick Fog, Haze; 0.20 cm of precipitation	Avg. 0 ℃; Completely Overcast, Fog, Windy; 0.25 cm of precipitation	Very Overcast, Fog, Haze; 1.02 cm of precipitation	02-11-2020
Avg. 0.56 ℃; Clear, Windy	Avg. 3.9 ℃; Partly Cloudy	Avg. 3.9 ℃; Pretty Overcast, Fog; 1.16 cm of precipitation	Very Overcast, Thick Fog; 0.76 cm of precipitation	02-25-2020
Avg. 3.9 ℃; Some Clouds	Avg. 7.8 ℃; Clear	Avg. 12 ℃; Clear, Windy NEPTM	Actively raining during sampling; Very overcast; 1.12 cm of precipitation	03-10-2020

[a] Single samples collected by Travis Luncan due to inclement weather.

Detailed weather data charts from the National Weather Service can be found in the Appendix D. Additional site observations, such as water clarity, flow and height can be located on YSI probe data sheets in the Appendix A.

3.2 Cowan Creek Sample Site and Method Blanks

The Cowan Creek (CCJKR) site as previously stated served as the control site for the investigation into the presence of benzotriazole and tolytriazole in the surface waters surrounding the Wilmington air park. Throughout the duration of the 2019/2020 sampling season, no analytes of interest were detected in any CCJKR samples. The figure below shows two chromatograms produced by any CCJKR sample. The top chromatogram is from 11132019-CCJKR-R1-A and the bottom chromatogram was produced from 03102020-CCJKR-R1-B.

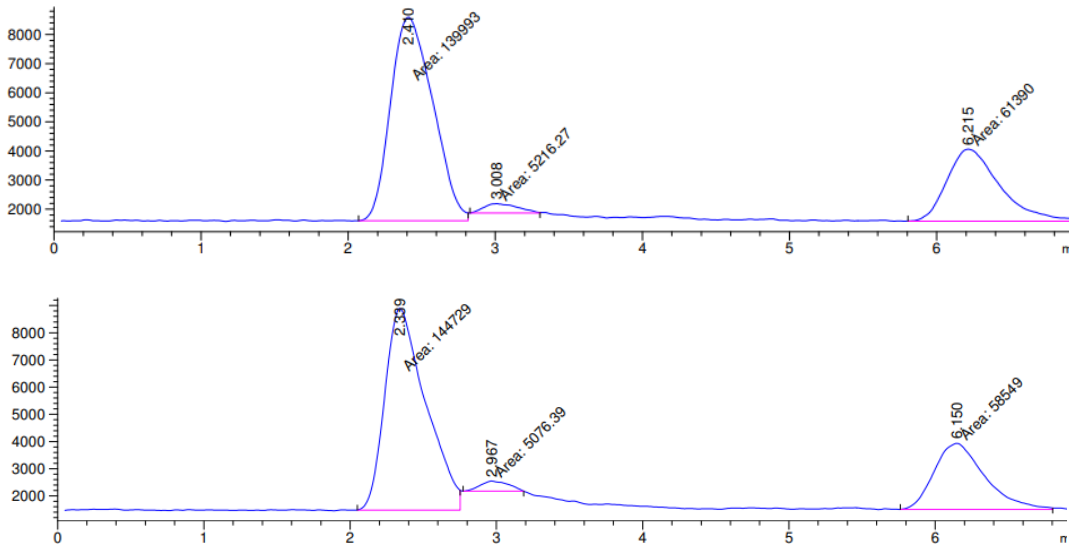

Figure 6 Typical chromatograms of CCJKR, top: 11132019-CCJKR-R1-A and bottom: 03102020-CCJKR-R1-B

Clearly, two peaks can be seen in both the above chromatograms at approximately the same time, with a similar shape and having approximately the same areas. The larger peak occurs at 2.410 minutes in 11132019-CCJKR-R1-A and at 2.339 minutes in 03102020-CCJKR-R1-B: a 2.95% difference. Additionally, these peaks differ by 3.27% in area. The smaller peak occurs at 3.008 minutes in 11132019-CCJKR-R1-A and at 2.967 minutes in 03102020-CCJKR-R1-B. The second peaks differ in elution time by 1.36% and in area by 2.68%. Unfortunately, these two

peaks are quite pervasive and appear in all samples that underwent the SPE process. All mock samples displayed the first extraneous peak near 2.3 minutes: two mock samples containing the peak can be seen below. The peak around 6 minutes is the internal standard.

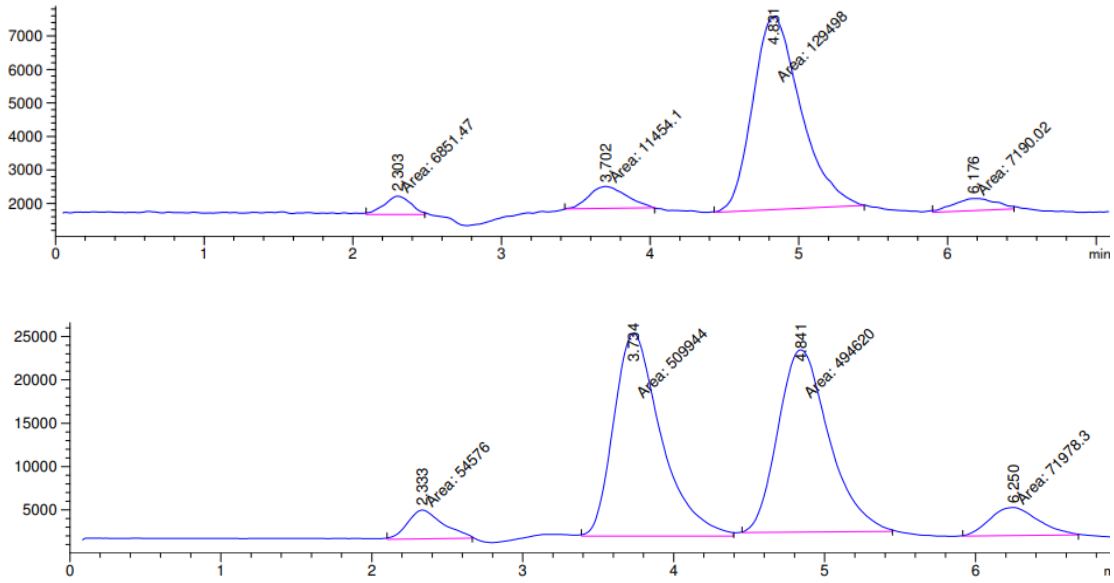

Figure 7 Example of mock samples, 01042021 -R2 (top) and 01152021-R1 (bottom)

Investigation into the potential source of the contaminant peaks included three method blanks, checking the 3% methanol in DCM solution and checking four different sources of LC-MS grade methanol. The three method blanks underwent the SPE process described in Section 2.2.2. The three method blanks were done on February 19th and March 4th and 5th 2021, they are shown below.

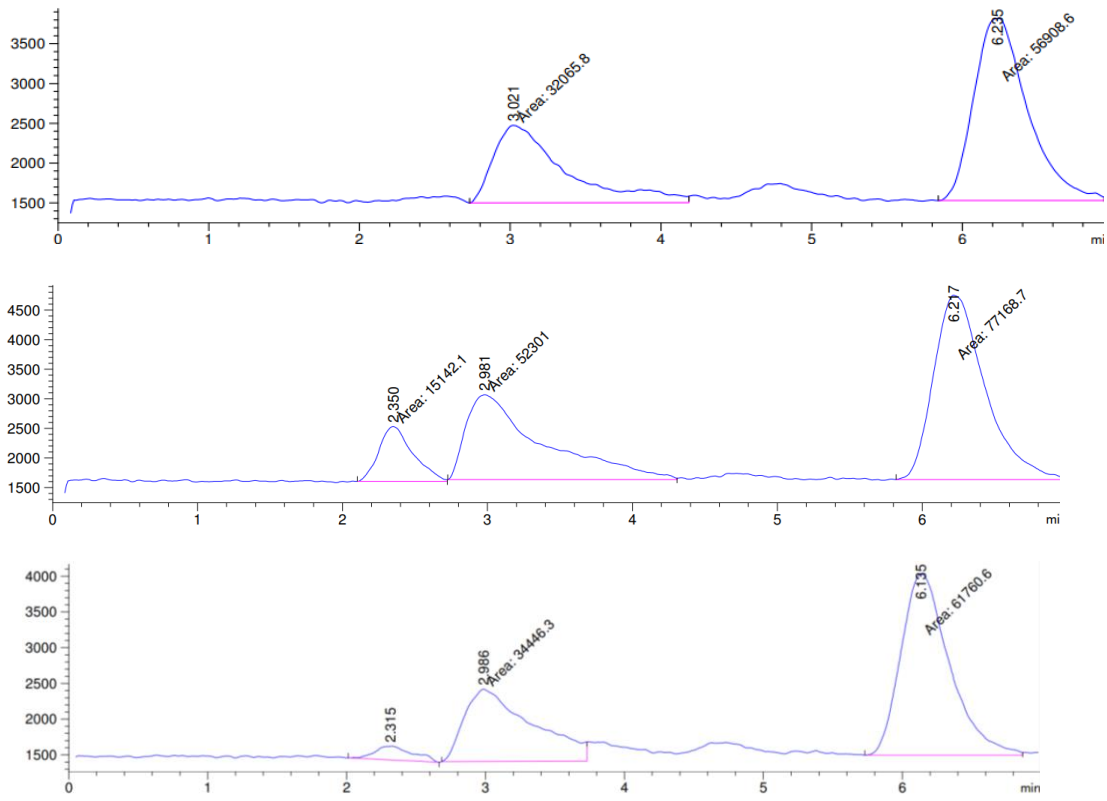

Figure 8 Method blank chromatograms: 02192021-Blank-HQ (top), 03042091-Blank-HQ (middle) and 03052021-Blank-HQ (bottom)

It can be seen that the peaks are more significant in the middle and bottom method blanks. Trying to source the origin of the contaminant peaks, 5 mL of 3% methanol in DCM was put into a centrifuge tube and blown down to dryness using nitrogen. The sample was then redissolved with 1 mL of methanol. This was also done with 5 mL of methanol containing 1 drop of the concentrated HCl utilized during the SPE procedure. The produced chromatograms can be seen below.

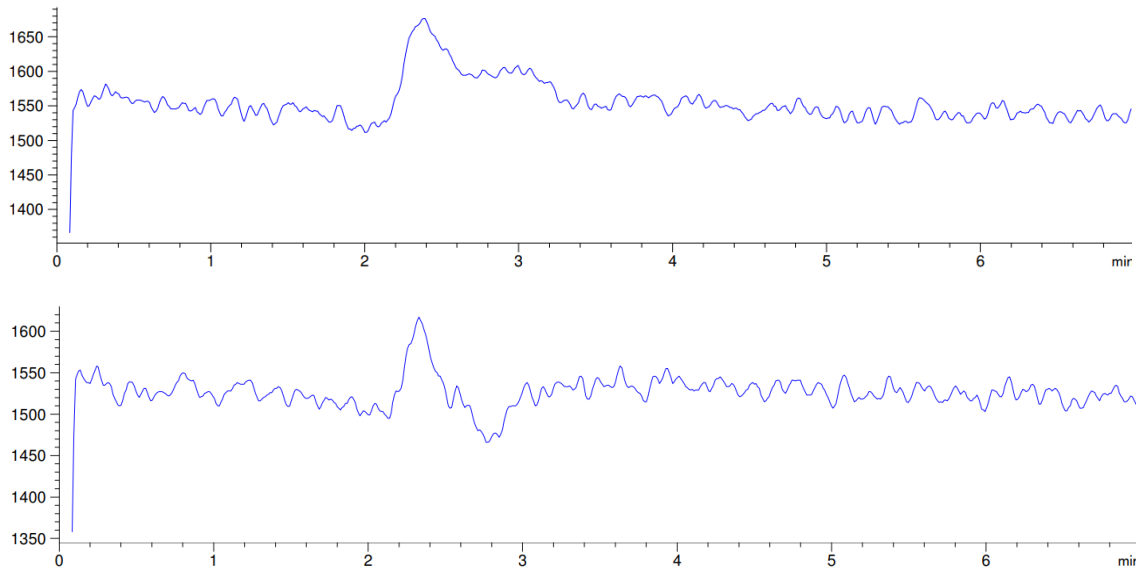

Figure 9 Investigating 3% MeOH in DCM, 02252021-DCM (top) and 03122021-HCl-MEOH (bottom).

The chromatograms produced seemed to not strongly indicate that DCM was the source of the contamination or the HCl. Both chromatograms do contain a "peak" near 2.3 minutes; however, it is very poorly defined and barely above baseline. Following this, four different samples of LC-MS methanol were procured. The methanol was sourced from: bench top bottle methanol used directly in SPE activities, two stock methanol bottles that have been used to fill the bench top bottle and methanol sourced from another lab entirely. These four methanols were analyzed without any treatment using the chosen LC-MS method (ran as instrument blanks) and produced no data of specific interest. These chromatograms can be seen in the Appendix B. After straight analysis of the methanols, 5 mL aliquots of each were placed into centrifuge tubes and blown down to dryness using nitrogen. The associated chromatograms are located below.

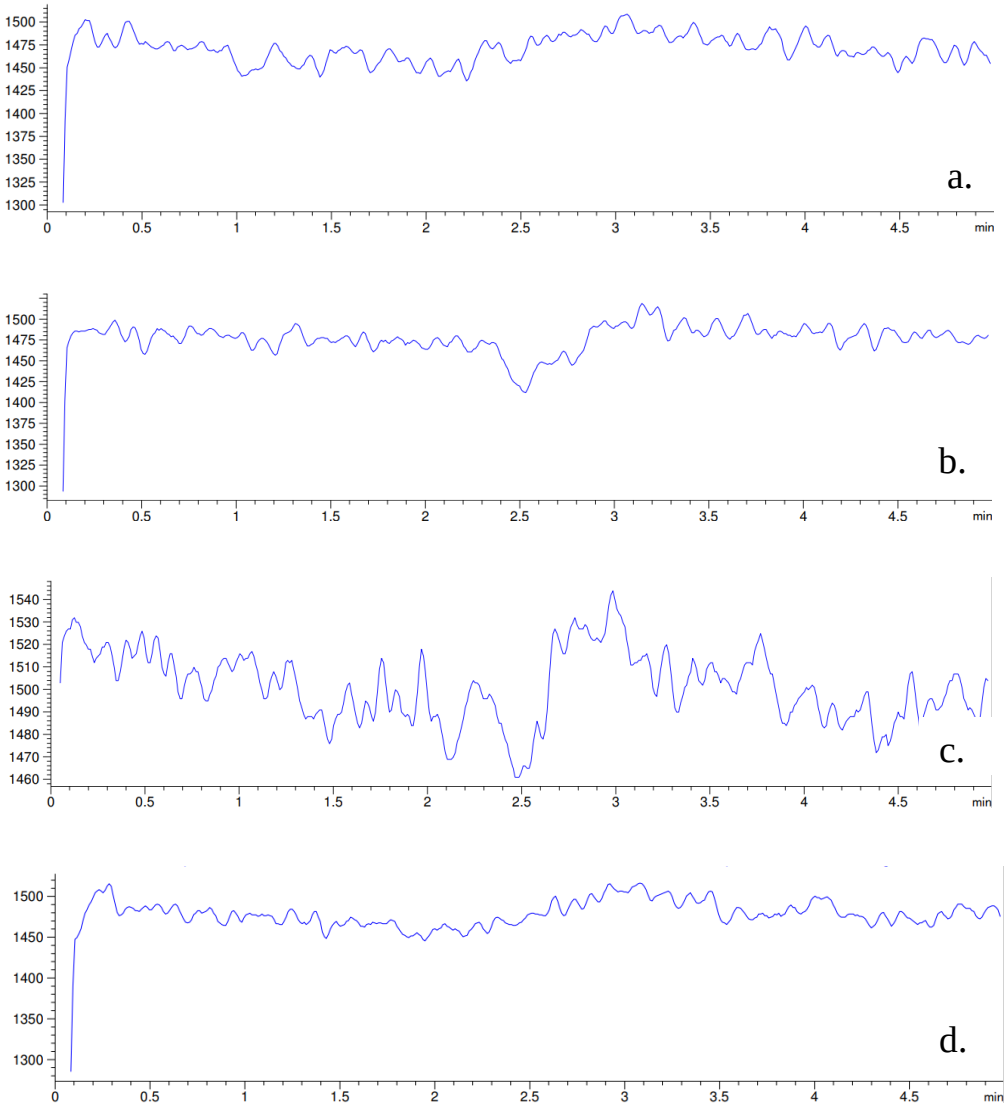

Figure 10 Investigating various MeOH sources, a. different lab b. bench top c. MeOH opened 0208221 d. MeOH opened 011221.

None of the four methanols analyzed appeared to incriminate themselves as a source of contamination. Given that the DCM, methanol and HCl analysis demonstrated no clear origin of the two contaminant peaks consistently appearing near 2.3 minutes and 3.0 minutes, the apparent option left was contaminate introduction during the SPE cartridge processing.

3.3 Indian Run and Lytle Creek Sample Site

Indian Run and Lytle Creek were determined to both contain detectable levels of TTZ; however, only Lytle Creek was characterized by measurable levels of BTZ. On three days BTZ was detected at measurable levels, other sample days Lytle Creek had BTZ concentrations at trace levels and had no detection on one sample day. A typical chromatogram produced by the Indian Run site is shown below.

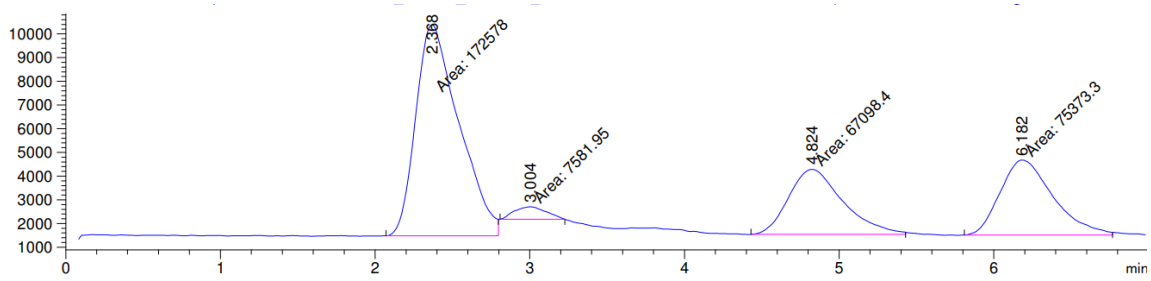

Figure 11 Typical Indian Run Site Chromatogram (02112020-IRJKR-R1-A-1).

The chromatogram in Figure 11, displays clearly a peak for tolytriazole (4.824 minutes) and the 5,6-dimethylbenzotriazole internal standard peak (6.182 minutes). Typical chromatograms for the Lytle Creek site are shown below and display: day with clearly detectable BTZ and TTZ levels; day with trace levels of BTZ and detectable levels of TTZ and day with no discernable BTZ but detectable TTZ.

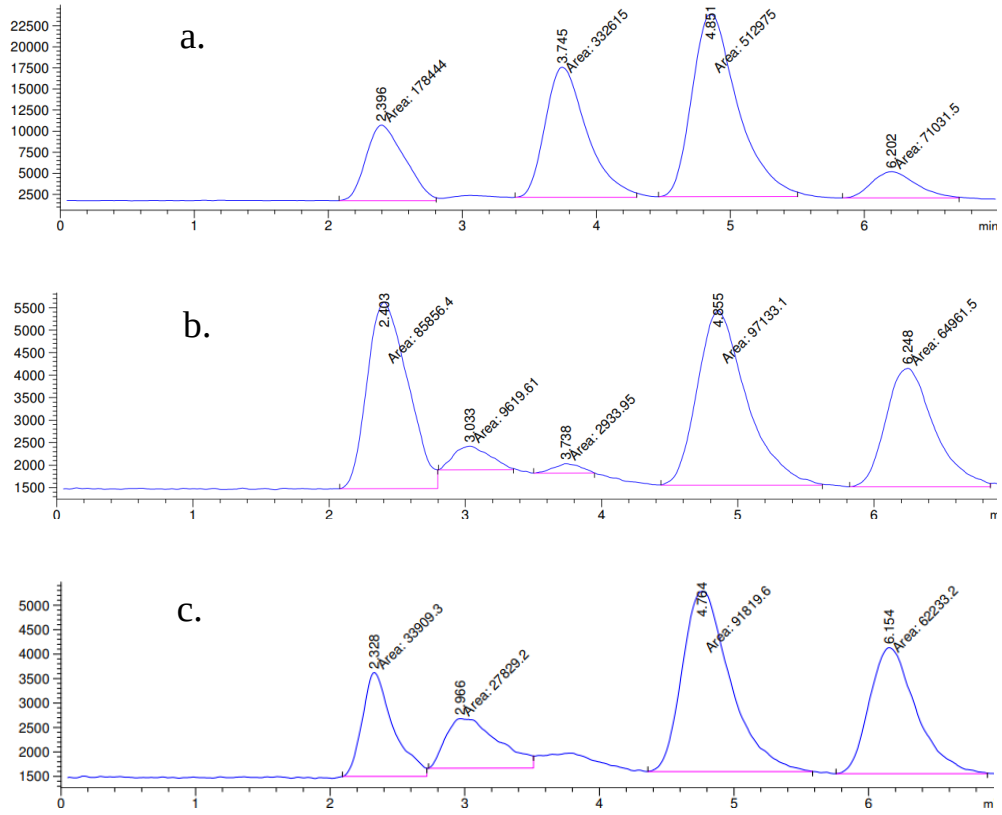

Figure 12 Example chromatograms for LCFR site: a. 11202019-LCFR-R2-A-1, b. 02252020-LCFR-R1-A-1, c. 03102020-LCFR-R1-A-1.

Chromatogram *a.* (11202019-LCFR-R2-A-1) in Figure 12 has clearly defined peaks and minimal appearance of the second contaminant peak most likely due to the scale of all other peaks. Chromatogram *b.*(02252020-LCFR-R1-A-1) shows a BTZ peak that is detectable but has immense interference from the second contaminant peak and is not resolvable. The third chromatogram, *c.* from 03102020-LCFR-R1-A-1, has a defined internal standard peak, TTZ peak and first contaminant peak. The second contaminant peak has the appearance of potentially blending into a very small BTZ peak, but since this BTZ peak is truly indiscernible it may just be tailing from either of the contaminant peaks.

MS peak areas, generated calibration equations (from three sets of standards) and percent recovery corrections were used to calculate the analyte concentrations present in all samples.

Table 13 contains the percent recovery for the 5,6-DMBTZ internal standard alongside standard. deviations and 95% confidence interval. These percent recovery values were used to correct observed analyte concentrations.

Table 13 5,6-Dimethylbenzotriazole percent recovery for all sample site replicates; including site/day average, standard deviation and 95% confidence interval.

Sample Day	Sample Site	R1-A	R1-B	R1-C	R2-A	R2-B	R2-C	Average	Standard Deviation	95% Confidence
11-13-2019	CCJKR	67.3	57.5	72.6	73.5	67.8	75.1	69.0	6.1	3.5
	IRJKR	74.1	86.4	76.3	68.2	73.9	71.9	75.2	5.9	3.4
	LCFR	71.0	65.7	72.8	Severely Broken[a]			69.8	3.4	2.7
11-20-2019	CCJKR	66.2	66.5	71.9	60.1	68.1	69.4	67.1	3.9	2.2
	IRJKR	66.9	75.1	71.4	72.1	34.0	92.7	68.7	18.3	10.4
	LCFR	86.7	77.1	70.5	66.1	73.4	70.0	74.0	6.9	3.9
12-04-2019[b]	CCJKR	77.5	78.2	76.5	Not Collected			77.4	1.3	0.85
	IRJKR	74.4	84.2	76.4				78.6	4.9	3.4
	LCFR	80.9	102	67.8				83.6	15.1	9.9
01-14-2020	CCJKR	78.3	77.1	80.2	76.0	88.2	88.5	81.4	5.4	3.0
	IRJKR	89.9	74.0	75.0	68.9	76.4	62.2	74.4	8.8	5.0
	LCFR	57.3	63.0	58.4	63.3	65.7	50.1	59.6	5.4	3.0
01-23-2020	CCJKR	69.9	77.2	81.7	87.5	68.0	72.9	77.9	7.4	4.0
	IRJKR	63.0	36.4	68.0	65.4	70.0	73.7	64.2	13.4	7.2
	LCFR	72.2	70.6	81.9	76.0	80.4	81.4	78.1	4.9	2.7
02-11-2020	CCJKR	53.7	62.5	37.4	52.5	60.3	76.2	57.1	12.3	7.0
	IRJKR	75.5	70.6	103	41.7	66.2	84.2	73.6	19.6	11.1
	LCFR	73.0	45.2	54.0	59.8	Lost[c]	52.1	56.8	9.9	6.1
02-25-2020	CCJKR	62.0	58.8	64.4	63.2	66.5	66.6	63.6	2.9	1.7
	IRJKR	79.2	73.0	74.1	71.2	77.1	80.2	75.8	3.5	2.0
	LCFR	62.0	64.0	75.7	73.7	68.2	72.0	69.3	5.3	3.0
03-10-2020	CCJKR	47.6	56.2	45.7	60.6	48.1	55.4	52.3	5.7	3.2
	IRJKR	59.1	47.1	53.1	93.9	70.7	61.2	66.3	16.7	9.4
	LCFR	59.3	59.6	60.5	59.1	66.0	86.5	65.2	10.3	5.8

a Sample contaminated by label.
b Single samples collected by Travis Luncan.
c Centrifuge vial broken.

The average percent recovery for all the samples was 70.0% with a standard deviation of 8.2 and a 95% confidence interval of 4.8. Average percent recoveries for sample sites ranged from 34.0-103%. R2 replicate bottles were not collected for sample day 12042019. A significant number of sample bottles fractured during thaw, even though they were thawed in a refrigerator with caps slightly unscrewed. Only one sample bottle (11-13-2019-LCFR-R2) was not processed

due to such an event, as it was severely broken. Bottles could typically be heard breaking within the first two hours of thawing. No significant difference was observed between fractured or unfractured sample replicates. A full list of fractured sample bottles can be found in Appendix C. Unfortunately, one replicate sample was lost (02112020-LCFR-R2-B) after completion of the SPE process during transfer from the centrifuge tube to the auto-sampler vial.

Samples usually presented very similarly after filtration; however, 0225-2020-IRJKR and 02252020-LCFR (more prominently) had a slight yellow tinge after sample filtration. The glass fiber filters through which approximately 300 mL of each replicate sample bottle was filtered can be seen in Appendix C alongside a more usual set of filters. LCFR collected little material and has a slight yellow beige color. IRJKR also appears to have a slight difference in shade compared to CCJKR filters.

The percent recoveries presented in Table 13 were used to calculate the concentration of analytes present in the original sample. Shown below in Table 14 the BTZ analyte concentrations are displayed for the Lytle Creek sample site, in addition to the corresponding percent recovery. Three sample days fell below the limit of detection and one sample day displayed no evidence of BTZ present in the sample.

Table 14 Determined BTZ concentrations for Lytle Creek: calculated BTZ concentration, internal standard recovery and corrected BTZ concentration.

Sample Day	BTZ Concentration (µg/L)	Internal Standard Recovery (%)	Corrected BTZ Concentration (µg/L)
11-13-2019[a]	0.437±0.02	69.8±3.4	0.626±0.022
11-20-2019	2.556±0.11	74.0±6.9	3.47±0.4
12-04-2019	0.123±0.02	83.6±15.1	0.148±0.01
01-14-2020	0.122±0.1	59.6±5.4	0.205±0.02
01-23-2020	<0.0501	78.1±4.9	<0.0501
02-11-2020[b]	<0.0501	56.8±9.9	<0.0501
02-25-2020	<0.0501	69.3±5.3	<0.0501
03-10-2020	<0.0501	65.2±10.3	<0.0501

[a] 11132019-LCFR-R2 severely broken during thawing and contaminated by label. Not processed.
[b] 02112020-LCFR-R2-B vial broken. Total 5 samples.
[c] Detection limit for BTZ was determined to be 0.0501 µg/L.

The limit of detection (LOD) for BTZ was 0.0501 µg/L. This was determined by successive dilutions of standards: when corrected for concentration of samples, the effective LOD was 0.0501 µg/L. Corrected concentrations were determined by dividing calculated concentrations by the respective percent recovery and dividing by 100 for dilution as the concentrated sample went from 100 mL down to 1 mL. An example of this can be seen below using 12042019-LCFR-R1-A sample data.

BTZ Standard generated Calibration Equation:

$y=1468x+3570 \qquad R^2=0.9994$

12042019-LCFR-R1-A MS Area:

19460.1

Calculated Concentration:

$$x=\frac{19460.1-3570}{1468}= 10.824 \text{ µg/L}$$

Corrected Concentration for Dilution and Percent Recovery:

$$\text{Corrected Concentration (µg/L)} = \frac{Calculated\ Concentration}{\frac{Percent\ Recovery}{100}x100}$$

$$\text{Corrected Concentration (µg/L)} = \frac{10.824\ \mu g/L}{\frac{77.992}{100}x100} = 0.138\ \mu g/L$$

These same calculations were done for all samples. Average concentrations were also corrected for concentration factor (100 mL original water sample to 1 mL final methanol sample). The average concentration of benzotriazole detected at the Lytle Creek sample site ranged from 0.148 µg/L to 3.47 µg/L. The three sample days below the detection limit, did have a small indication of a BTZ peak, but the peaks were so slight and contained too much interference from the unknown contaminant peaks that the concentration could not be determined in confidence. An example of this instance can be seen in Figure 12 *b.* of 02252020-LCFR-R1-A-1.

Tolytriazole was detected at both Lytle Creek and Indian Run on every sample day at measurable levels. TTZ concentrations were calculated in the same manner as BTZ concentrations and corrected to maximums in original sample. These concentrations can be seen below in Table 15 for Lytle Creek and Indian Run.

Table 15 Determined tolytriazole concentrations for each sample site and day: calculated TTZ concentration, internal standard recovery and corrected TTZ concentration.

Sample Day	Sample Site	TTZ Concentration (µg/L)	Internal Standard Recovery (%)	Corrected TTZ Concentration (µg/L)
11-13-2019	IRJKR	4.316±0.02	75.2±5.9	5.66±0.45
	LCFR[a]	8.348±0.27	69.8±3.4	12.0±0.24
11-20-2019	IRJKR	1.042±0.03	68.7±18.3	1.67±0.71
	LCFR	3.726±0.09	74.0±6.9	5.07±0.38
12-04-2019	IRJKR	0.282±0.02	78.6±4.9	0.359±0.01
	LCFR	4.000±64.1	83.6±15.1	4.82±0.26
01-14-2020	IRJKR	0.503±0.04	74.4±8.8	0.680±0.05
	LCFR	0.101±0.08	59.6±5.4	0.170±0.10
01-23-2020	IRJKR	0.991±0.06	64.5±13.4	1.67±0.65
	LCFR	1.287±0.05	78.1±4.9	1.65±0.06
02-11-2020	IRJKR	0.508±0.10	73.6±19.6	0.706±0.08
	LCFR[b]	0.155±0.22	56.8±9.9	2.76±0.16
02-25-2020	IRJKR	0.162±0.74	75.8±3.5	0.214±0.01
	LCFR	0.935±0.13	69.3±5.3	1.35±0.13
03-10-2020	IRJKR	1.186±0.25	66.3±16.7	1.81±0.10
	LCFR	0.467±0.02	65.2±10.3	0.725±0.06

[a] 11132019-LCFR-R2 severely broken during thawing and contaminated by label. Not processed.
[b] 02112020-LCFR-R2-B vial broken. Total 5 samples.
[c] Detection limit for BTZ was determined to be 0.0501 µg/L.

Analyte concentration levels were higher than anticipated. The Lytle Creek sample site usually had a substantially higher TTZ concentration than the Indian Run site. Concentration ranges were 0.725-12.0 µg/L and 0.214-5.66 µg/L for Lytle Creek and Indian Run, respectively. The highest concentration observed simultaneously for both sites was November 13th, 2019. The lowest concentration observed for Lytle Creek site was March 10th, 2020 and February 25th for Indian Run. This occurrence of Indian Run having a higher TTZ concentration than Lytle Creek is odd considering that the Lytle Creek site appeared high for five of the seven other sample days; however, it is possible that the Indian Run water treatment site was in use rather than the Lytle Creek water treatment site. Additionally, during the March 10th sample day it was actively raining and may have caused additional run off or leeching from contaminated surrounding soil. Table 16 below displays the average TTZ analyte concentrations present at LCFR and IRJKR for the highest and lowest days with corresponding weather data.

Table 16 Weather data for high and low extrema of LCFR and IRJKR along with TTZ analyte concentration.

Extrema	Sample Site	Sample Day	TTZ Analyte Concentration (µg/L)	Ambient Temperature(℃)	Precipitation[26] (cm)
Highest	LCFR	11-13-2019	12.0±0.2	-10	0[a]
	IRJKR	11-13-2019	5.66±0.45	-8.89	
Lowest	LCFR	03-10-2020	0.725±0.06	12.2	1.12
	IRJKR	02-25-2020	0.214±0.01	5	0.76

[a] Snow was on the ground, photo for this date can be seen in Appendix D.

Despite the weather data claiming that there was no snow in the preceding days leading up to 11-13-2019, there was 0.51 cm of precipitation in the prior two days and there was snow on the ground. Precipitation was originally reported in inches and converted to centimeters. A photo of taken on 11-13-2020 of a field near IRJKR can be seen in Appendix D. When comparing detected concentration ranges to the weather conditions proceeding the sample day and the day of sample, the results appear reasonable. SAE International, an aerospace company, has a long list of specifications for aircraft de-icers and for the conditions and modes of use that are used by the Federal Aviation Association (FAA). There are four different categories of aircraft de-icing fluids labeled Type I through Type IV. Type I fluids are low viscosity fluids containing glycols (usually ethylene or propylene) and are comprised of one percent or less of additives. Type II, III, and IV are composed similarly but typically contain more additive up to two percent. Type II and Type IV have higher viscosities than Type I and Type III. Type III has properties that are in between Type I and Type II and IV. Larger airlines more prevalently use Type I and Type IV in combination while smaller airports with smaller airlines use Type I and Type II. [27],[28] According to the FAA, planes must be de-iced when there is potential for ground icing (10 ℃ with precipitation) and have anti-icing fluids applied when the outside air temperature is 10 ℃ (50 °F) and no precipitation so that icing does not occur at higher altitudes after takeoff. Looking at the YSI probe data sheets, it can be seen that by these guidelines that de-icing fluids and anti-

icing fluids would have been applied during all sample days except for March 10th, 2020. March 10th, 2020 had an ambient temperature of 12.2 ℃ and the proceeding three days had the highest recorded temperatures. This sample day had the lowest recorded analyte of interest concentrations observed during the investigation. No BTZ was observed and maximum TTZ concentrations for LCFR and IRJKR were both less than 1 µg/L. Additionally, the sample day with the lowest recorded temperature was November 13th, 2019 (around -10 ℃) would certainly have had the fluids applied day of sampling and in the preceding three days during which precipitation and low temperatures were recorded (below 10 ℃). For this sample day, the TTZ maximum concentration for LCFR and IRJKR were almost 12 µg/Land almost 6 µg/L respectively.

3.4 2019, 2019/2020 Sampling Seasons and 2021 Continuation

Both the 2019 and the 2019/2020 sampling seasons had their respective difficulties; however, both sampling seasons were afflicted with the appearance of an unknown contaminant peak. These peaks appeared in all samples prepared by the SPE process and analyzed by LC-MS in both project years. An example of the unknown peak in the chromatograms for the 2019 sampling season conducted by Jessica Wiese is shown below in Figure 13 alongside a comparison chromatogram from the 2019/2020 season.[22]

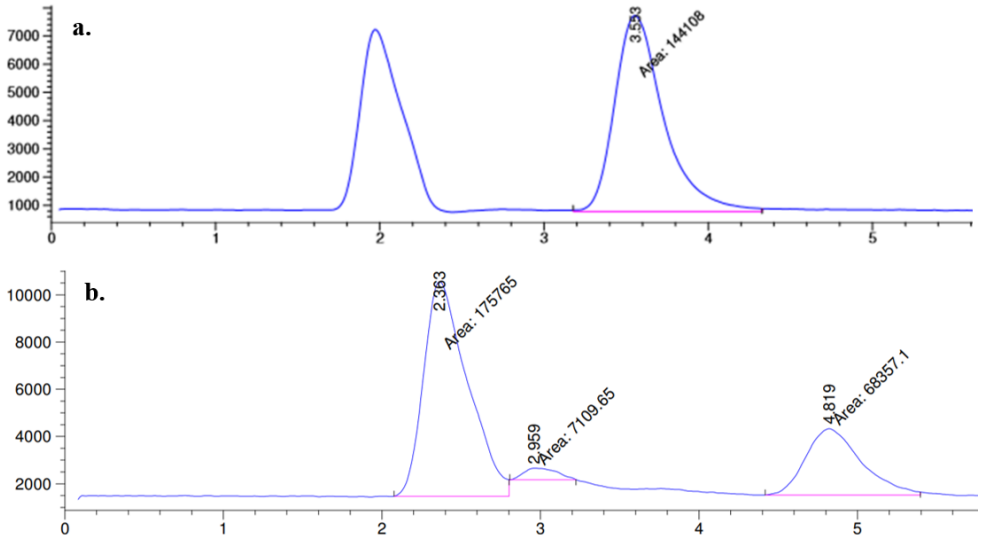

Figure 13 Comparison of unknown contaminant peak, Indian Run site: (a.) 02-01-2019 and (b.) 02-11-2020

Both *a.* and *b.* display a contaminant peak and a TTZ peak; however, *b.* displays a second previously discussed contaminant peak. This secondary peak was also seen in the 2019 study, but generally only under "Full Scan" conditions. The 2019 sampling season investigated JP-8 jet fuel as a potential source of the contaminant, as there was evidence that there could be hydrocarbon fragmentation. The 2019/2020 investigation concluded that the unknown contaminant peaks are actually due to the SPE process, considering that the peaks were also present in the method blanks. The following sampling season should further explore this process as a potential source. If the same SPE cartridges are used, a change in elution solvent or the conditioning process may be the only available routes to explore as options to ameliorate this obstacle.

The 2019 and 2019/2020 sampling seasons had similar percent recoveries. The iteration of the project resulted with a general range of 70-80% internal standard recovery. The average of the reported percent recoveries was approximately 64% for the 2019 season. The second iteration of the project had a similar general range of percent recoveries and an average recovery of approximately 68%.

One of the future goals established by the 2019 sampling season was to develop a method that would better separate BTZ and TTZ peaks. Additionally, there was a desire for the two isomers (4-methyl-benzotriazole and 5-methyl-benzotriazole) to be separated into two peaks. The initial goal of better BTZ and TTZ peak separation was accomplished by slightly changing the flow rate and eluent ratio. The second goal was not able to be achieved. Slight isomer separation was indeed observed, but only under LC-MS conditions using a flow rate of 0.120 mL/min and an eluent mix consisting of greater than forty-five percent 0.1% formic acid in water and less than fifty-five percent 0.1% formic acid in methanol. Isomer separation began under these conditions but were in no way resolvable and different instrumentation may be needed to achieve this goal. Exploring a different column length may be a pathway to achieve this ambition.

Site data presented similarly in both the 2019 and 2019/2020 sampling seasons. Table 17, below, displays the analyte concentration ranges for both investigative years in relation to the site at which they were observed.

Table 17 Comparison of analyte concentrations between sampling years

Site	BTZ 2019 (µg/L)	BTZ 2019/2020 (µg/L)	TTZ 2019 (µg/L)	TTZ 2019/2020 (µg/L)
IRJKR	Below LOD	Below LOD	0.111-1.248	0.214-5.66
LCFR	Below LOD	0.148-3.47	0.822-3.435	0.725-12.0

Neither investigative years observed any analytes of interest at the control site Cowan Creek. The 2019 sampling season detected BTZ at both Indian Run (IRJKR) and Lytle Creek (LCFR) but the levels fell below the limit of detection (LOD). The 2019/2020 sampling season detected quantifiable levels of BTZ at LCFR (0.148-3.47 µg/L) and potentially at IRJKR but quantifiable due to interference and potentially below the LOD. LCFR and IRJKR had concentration ranges

of 0.822-3.435 µg/L and 0.111-1.248 µg/L respectively in the 2019 season. The 2019/2020 sampling season saw a significant increase the maximum: LCFR and IRJKR were observed to have ranges of 0.725-12.0 µg/L and 0.214-5.66 µg/L respectively. The maximum percent increase between the first investigation and second investigation for tolytriazole concentrations were 248% and 354% for Lytle Creek and Indian Run, respectively.

An article by *Shi et al.* used hazard quotients (HQ) in their environmental risk assessments regarding benzotriazoles. HQ is derived from the ratio of average environmentally detected concentrations and the predicted not effect concentrations (PNEC). HQs fall into three different risk levels: low risk (<0.01), medium risk (≥0.1) and high risk (≥1). The average detected concentration of BTZ during the 2019/2020 sample season was 2.07 µg/L at the Lytle Creek site. *Shi et al.* determined the PNEC of BTZ to be 15.8 µg/L. *Shi et al.* has determined PNECs for the 4- and 5-methyl TTZ isomers, but not TTZ. However, 4- and 5-methyl TTZ isomers have PNECs of 21.0 and 5.52 µg/L, respectively and can be used to give a maximum HQs from the maximum detected TTZ concentration.[29] Using the calculated average BTZ concentration at Lytle Creek and the PNEC provided by *Shi et al.* the HQ for BTZ would be 0.131. This would indicate that the levels of BTZ detected at Lytle Creek would be medium environmental risk and may be a cause for concern. The maximum TTZ concentration detected was 12.0 µg/L for the 2019/2020 sample season. Using the PNEC for 4-MBTZ and 5-MBTZ the maximum HQ for either isomer would be 0.59 and 2.16, respectively. The maximum concentration of TTZ at Indian run was 5.66 µg/L and would result in HQs of 0.270 and 1.03. It would be reasonable to assume that the HQ for TTZ would be in between these values and would indicate that the TTZ HQ would all into the medium to high risk level. Acute toxicity may not be

a concern now, but concern is still merited with regard to the potential long-term effects on aquatic plants and organisms.

4. Conclusions

The 2019/2020 investigation into the appearance of benzotriazole and tolytriazole in the streams surrounding the air park in Wilmington, Ohio included a number of aims and goals. The primary goal of this year's project continuation was to monitor and compare the results to previous findings. Secondary goals included optimizing the LC-MS method to separate and resolve analyte peaks and identifying the source of the unknown contaminant peak. An additional aim was to see if there was an observable correlation between weather and detected analyte concentration levels.

The findings in the 2019 investigation determined that there were trace concentrations of benzotriazole in the Indian Run and Lytle Creek samples, but levels fell below the LOD. The 2019/2020 investigative study detected benzotriazole in all Lytle Creek samples on multiple sample days. Three sample days contained trace levels of benzotriazole, and three sample days produced measurable benzotriazole levels. Benzotriazole levels for the Lytle Creek site ranged from 0.148-3.47 µg/L. Measurable levels of tolytriazole were present in both the aforementioned sample sites on all sample days. Concentration ranges of tolytriazole were 0.822-3.435 µg/L and 0.111-1.248 µg/L for Lytle Creek and Indian Run sample sites respectively in the 2019 study. The concentration ranges for TTZ in the 2019/2020 study were 0.725-12.0 µg/L for Lytle Creek and 0.214-5.65 µg/L for Indian Run. The maximum percent increases between the first investigation and second investigation for tolytriazole concentrations were 248% and 354% for Lytle Creek and Indian Run, respectively. Neither sampling year detected tolytriazole or benzotriazole in any of the Cowan Creek samples. When considering the potential increase in air traffic due to the presence of an online retailer, and thusly an increase in onsite use of de-icing and anti-corrosive fluids, the results displaying an increase in detectable analyte concentrations

compared to the previous year's findings are unsurprising. This year's findings indicate the Indian Run water treatment beds have been reactivated. Additionally, the concentrations detected do not pose an acute toxicity threat to aquatic or terrestrial life at their current levels according to the studies and findings presented previously. However, even at these medium HQ level these compounds may pose some small threat to the typical functions of plant life and potentially could be found in their tissues.

Analyte concentrations determined to be present in the samples collected could not be as clearly correlated to weather events and conditions as the previous year. The previous sample year found that the highest concentrations of tolytriazole occurred on the sample day with the lowest ambient temperature and the most amount of precipitation. The lowest concentrations were found on the sample day with the highest ambient temperature and a decent amount of precipitation. The 2019/2020 investigation observed the highest concentration on tolytrizole on the day with the lowest temperature but with no recorded precipitation despite there being ground snow cover present (11/13/2019). The lowest concentration of tolytriazole determined were on different days. The lowest recorded concentration of tolytriazole at the Lytle Creek site was on 03/11/2020 when the temperature was warmest and there was precipitation. The lowest recorded concentration for the Indian Run site was on 02/25/2020 when the temperature was still below 10 ℃ and there was little precipitation. It seems that the highest concentrations can be correlated to the lowest temperatures, but the lowest concentrations cannot be linked to any specific weather condition. This could be potentially because there is no way to tell consistently how long runoff is held in the treatment beds at the two treatment facilities before being released. One treatment facility may release runoff quicker than the other depending on need and if conditions permit.

Relying on the number reported in June 2019, flights per day increased by approximately 130% going from an average of 6 flights per day to an average of 14.[22] When compared to the maximum percent increase of TTZ concentrations between sampling seasons (248% and 354% for Lytle Creek and Indian Run respectively), two options appear: the efficiency of analyte removal has decreased due to increased air traffic or the air traffic increased beyond an average of 14 flights per day. However, limits in information and discrepancies between span of sampling seasons make such a conclusion difficult to speculate. Due to the popularity of the online retailer, it seems most likely that the average number of flights per day exceeded 14 from the air park: especially towards the beginning of the winter holiday season when people's shopping needs increased. It is also unknown whether the air park utilizes fluids containing the analytes of interest according to daily weather conditions or if they are continuously used throughout the season once weather first demands.

The 2019 and 2019/2020 sample years had additional similarities such as the presence of a contaminant peak in all samples; however, the 2019/2020 sampling year appeared to have a second contaminant peak. This peak could potentially be a second contaminant, or it could be a resolved peak separated from the primary contaminant peak due to the change in LC-MS method. The previous years inquiry into the contaminant source concluded that it could potentially be due to the presence of JP-8 fuel in the samples. However, the 2019/2020 study identified these peaks in method blanks in addition to the site samples. These finding in addition to excluding solvents used during sample processing lead the 2019/2020 study to the conclusion that the contaminant peaks are derive from the cartridges employed in the SPE process.

The identification of the likely source of the contaminant peaks was one the few main goals for this second-year project continuation. A second more paramount goal for the

2019/2020 investigation was to ameliorate the LC-MS method to better separate the analytes of interest and attempt to separate and resolve the isomer tolytriazole peaks. The LC-MS method was augmented by slightly adjusting the elution rate and the eluent ratio. These slight changes contributed to better analyte separation within samples found to contain both benzotriazole and tolytriazole. The separation and resolution of the two methylated isomers was not manageable and may be beyond the capabilities of the LC-MS system available.

The continuation of this project for a third additional year could produce more data useful in protecting and monitoring the surround stream health. A consideration for furthering the project could be to looking into additional areas in which the analytes of interest could be found. Sediment analysis may be of particular interest considering their potential mobility due to their respective K_{oc} and K_{ow}. A primary interest to investigate in future project continuation is the potential presence of BTZ and TTZ degradates. This may be done by procuring potential degradate standards and analyzing them to find elution times for comparison. Additionally, keeping a similar sampling season timeline, similar to that of this study, may be advisable to allow for a similar perspective.

APPENDIX A

STANDARD OPERATING PROCEDURE 13.0

CALIBRATING A YSI PRO PLUS MULTIMETER

FOR PH, CONDUCTIVITY, AMMONIUM AND DO

AND OBTAINING FIELD MEASUREME

NTS

September 30, 2013

By

Shannon Hennelly, Anna Foote, Megan Huddleson,

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Revised

September 11, 2017

1. Scope and Application

The YSI Pro Plus meter is a remote sampling meter used to acquire water-monitoring data instantly at a remote sampling site. Coupled with a Quatro cable the YSI meter can measure four parameters simultaneously. This method explains how to properly calibrate the four external sensors used in the sampling of the Glen Helen Nature Preserve: pH, DO, conductivity and ammonium. Each sensor must be correctly calibrated before being employed during field sampling.

This method also explains the correct sampling technique and the proper logging of field data both with the YSI multimeter and student notebooks.

2. Summary of Method

This method explains calibration of the YSI multimeter and sampling protocols.

3. Health and Safety

All six standards used have NFPA Codes of zero for health, reactivity, and flammability. Some of the pH standards may cause irritation to the eyes and skin. It is best to wear appropriate personal protective equipment (PPE) at all times while in the lab to avoid contact with the eyes and to avoid prolonged exposure to the skin. This includes lab coat, nitrile gloves, and safety glasses at a minimum in addition to long pants and closed toe shoes.

4. Equipment and Supplies

4.1. YSI Multimeter:

4.1.1. YSI Pro Plus Meter

4.1.2. YSI Quatro Cable

4.1.3. Four Sensor Probes (pH, DO, Conductivity, Ammonium)

4.2. YSI Storage Container (screw-on plastic cylinder)

4.3. YSI Field Cover (metal cover)

4.4. YSI Transport Container (grey rubber sleeve)

4.5. Craftsmen Carrying Case

4.6. Log Book

4.7. Student Notebooks

5. Reagents and Standards

5.1. Deionized Water (DI)

5.2. Conductivity:

5.2.1. YSI 3161 Conductivity Calibrator Solution (1000 µS/cm ± 0.50% at 25°C)

5.3. Confidence Solution

5.3.1. YSI 5580 Confidence Solution

5.4. pH:

5.4.1. YSI 3821 Buffer Solution pH 4.00±0.01 at 25°C

5.4.2. YSI 3822 Buffer Solution pH 7.00±0.01 at 25°C

5.4.3. YSI 3823 Buffer Solution pH 10.00±0.01 at 25°C

5.5. Ammonium:

5.5.1. YSI 3841 1mg/L NH_4^+ -N Standard

5.5.2. YSI 3843 100mg/L NH_4^+-N Standard

KEYPAD

Number	*Key*	*Description*
1		**System** Opens System Menu from any screen. Use to adjust system settings.
2		**Sensor** Opens Sensor Menu from any screen. Use to enable sensors and display units.
3	Cal	**Calibrate** Opens Calibrate Menu from any screen. Use to calibrate all parameters except temperature.
4		**File** Opens File Menu from any screen. Use to view data and GLP files, set up site and folder lists, and delete data.
5		**Backlight** Press to turn the instrument backlight on and off and to adjust the display contrast when pressed with the left or right arrow key.

Number	Key	Description
6		**Right Arrow** Use to navigate right in alpha/numeric entry screens. Can be pressed simultaneously with Backlight key to increase display contrast.
7		**Down Arrow** Use to navigate through menus and to navigate down in alpha/numeric entry screens.
8		**Power** Press to turn the instrument on. Press and hold for 3 seconds to turn off.
9	?	**Help** Press to receive hints & tips during operation.
10	ENTER	**Enter** Press to confirm selections, including alpha/numeric key selections.
11		**Left Arrow** Use to navigate left in alpha/numeric entry screens. Press to return to previous menu in all screens except alpha/numeric entry. Can be pressed simultaneously with Backlight key to decrease display contrast.
12	Esc	**Exit/Escape** Exits back to Run Screen. When in alpha/numeric entry screen, escapes to previous menu.
13		**Up Arrow** Use to navigate through menus and to navigate up in alpha/numeric entry screens.

6. Calibration Procedure

6.1. Dissolved Oxygen:

6.1.1. Insert the Quarto probe into a saturated storage container (make sure sponge is moist)

6.1.2. Push <Cal> to calibrate, select <DO>

6.1.3. Press <DO%>

6.1.4. Once % DO and temperature stabilize to slightly <100% press enter to "accept calibration".

6.1.5. Click <Cal> to finish.

Note: This is more of a *check* than an actual calibration.

6.2. Conductivity

6.2.1. Fill one beaker with high quality to use for washing.

6.2.2. Fill another beaker with enough conductivity solution (5.1.1) to be able to completely cover the conductivity probe (the conductivity probe is the black one with the metal prong extending out of the tip)

6.2.3. Remove the Quatro from the storage container and rinse with high quality water then gently shake dry.

6.2.4. Submerge completely in the conductivity stock standard for conductivity.

6.2.5. Press <CAL> for calibration, select "Conductivity"

6.2.6. Press the <Enter> button

6.2.7. Select specific conductance ("Sp. Conductance") and press <Enter>.

6.2.8. Select "SPC-µs/cm" for the units.

6.2.9. Click <Enter> for calibration menu.

6.2.10. Once the meter readout stabilizes, press <Enter> to "Accept Calibration"

6.2.11. Click <Enter>. Select User Field 1: Glen Helen.

6.2.12. After the probe calibrates rinse with DI water and store the probe in the clear plastic cylinder tube.

6.3. Confidence Solution

6.3.1. Submerge Quarto probe into confidence solution.

6.3.2. Press <CAL> for calibration, select “Conductivity”

6.3.3. Press the <Enter> button

6.3.4. Select specific conductance (“SP. Conductance”) and press <Enter>.

6.3.5. Select “SPC-µs/cm” for the units.

6.3.6. Click <Enter> for calibration menu.

6.3.7. Once the meter readout stabilizes, press <Enter> to ”Accept Calibration”

6.3.8. Press <Cal> to finish and after the probe calibrates, rinse with water.

6.3.9. Store the probe in the clear plastic cylinder tube.

6.4. pH

6.4.1. The standards for pH (5.3) can be diluted 50:50 with high quality water. This is because they are buffer solutions which means they are resistant to pH change.

6.4.2. Make about 100 mL each in labeled and DI cleaned beakers.

6.4.3. Put high quality water in another beaker to use for washing.

6.4.4. Remove probe from container and rinse with high quality water and gently shake dry.

6.4.5. The pH probe is the gray one with the rounded glass electrode on the tip. Submerge it completely in the first pH stock solution (pH 4).

6.4.6. Press <CAL> for calibration, select “ISE2 pH” and press the <Enter> button.

6.4.7. Click <Enter> to show the calibration menu.

6.4.8. Once the meter readout stabilizes, press enter to “Accept calibration”, click <Enter>.

6.4.9. The meter will then say “ready for point 2”.

6.4.10. Rinse the probe and place into the next buffer (pH 7) and repeat the same procedure.

6.4.11. After stabilizing and pressing <Enter> the probe will ask for point 3.

6.4.12. Rinse and place the probe in the last buffer (pH 10). Again let the readout stabilize and press <Enter> to “accept calibration”.

6.4.13. The probe will then ask for a fourth point, ignore this as only three are necessary.

6.4.14. Press <Cal> to finish and after the probe calibrates, rinse with water.

6.4.15. Store the probe in the clear plastic cylinder tube.

6.5. Ammonium

6.5.1. Pour about 50-100 mL of both ammonium standards (5.4) into two separated cleaned and labeled beakers.

6.5.2. Put high quality water in another beaker to use for washing.

6.5.3. Remove probe from container and rinse with high quality water and gently shake dry.

6.5.4. The ammonium probe is the gray one with the flat buttom. Submerge it completely in the first NH_4^+ solution (1 mg L^{-1})

6.5.5. Press <CAL> for calibration, select "ISE2 NH4" and press the <Enter> button.

6.5.6. Click <Enter> to show the calibration menu.

6.5.7. Once the meter readout stabilizes, press enter to "Accept calibration", click <Enter>.

6.5.8. The meter will then say "ready for point 2".

6.5.9. Rinse the probe and place into the next ammonium solution (100 mg L^{-1}) and repeat the same procedure.

6.5.10. After stabilizing and pressing <Enter> the probe will ask for point 3, ignore this as there are only two.

6.5.11. Press <Cal> to finish and after the probe calibrates, rinse with water.

6.5.12. Store the probe in the clear plastic cylinder tube.

6.6. After the multimeter is calibrated fill out the Log Book with today's date and sign it.

7. Preparing Probe for Field Sampling

7.1. Once probe is calibrated then it is ready to take out into the field.

7.2. Remove from storage container and switch to metal sampling cage.

7.3. Put about 5 mL of DI water into the protective rubber sleeve

7.4. Slide the sleeve over the probe.

7.5. The probe will remain in the rubber sleeve just prior to sampling

8. Sample Collection and Logging Field Samples

8.1. Remove the rubber sleeve.

8.2. Gently submerge perpendicular to water flow (one person holds probe, one holds meter, all others write down the measurements as they are read aloud in their notebook/spreadsheet). Probe should now be submerged into water.

8.3. Have the person holding the meter read aloud the values from YSI read out.

8.4. Another person in the group will record the readings on the data sheet.

9. Reference

YSI *Professional Plus*. User Manual. **2009**

Standard Operating Procedure

WILMINGTON AIR PARK RUNOFF WATER SAMPLING PLAN

January 23, 2021

Audrey McGowin, PhD

Jessica Wiese

Lee A. Raska

A. SCOPE AND APPLICATION

1H-benzotriazoles are complexing agents that are widely used as anti-corrosives, engine coolants, aircraft de-icers, anti-freezing liquids, and silver protection in dishwashing agents. Chemically, 1H-benzotriazoles are soluble in water, resistant to biodegradation, only partially removed in wastewater treatment, and have the potential to pass drinking water treatment. Most benzotriazole (BTZ) compounds and their analogs are polar and thermally labile. In addition, BTZ are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTZ and BTZ analogs are deposited. The procedures outlined in this SOP were created for the collection of surface and ground water samples near Wilmington Air Park.

B. SUMMARY OF METHOD

The purpose of this Standard Operating Procedure (SOP) is to establish a procedure for the collection of surface and ground water samples near Wilmington Air Park in order to determine the presence of 1H-benzotriazoles, tolytriazoles, and comparable analogs in runoff from the airport's wastewater treatment plants.

C. HEALTH AND SAFETY

The analyst must assume that all surface and ground water samples are potentially contaminated and should be treated accordingly. Personal protection equipment (PPE) should be worn at all times while out in the field; this includes long sleeves, protective gloves, safety glasses, long pants and closed-toe shoes.

D. SAFETY AND CAUTIONS

1. Sample containers must be labeled according to the Sample Labeling Scheme outlined in Section F of this SOP.

2. During on site testing and sample collection, personnel must wear protective gloves and safety glasses.

3. Do not pour any reagents on the ground or into the water. Collect all waste materials for proper disposal in the lab in appropriately labeled waste containers.

4. Hiking boots and a raincoat are recommended for days when precipitation is possible.

E. EQUIPMENT AND SUPPLIES

1. Sampling protocol with Standard Sampling Form

2. Clipboard and laboratory notebook with ink pen

3. Clean amber glass bottles (500 mL) with PTFE-lined closures

4. Permanent marker for sample labeling

5. One small cooler with cool packs for sample preservation

6. Paper towels with Ziplock® bags

7. Rinsing bottle containing ASTM Type I water

8. YSI Multi-meter, pre-calibrated in the lab; DO, temperature, conductivity, pH

9. Waste containers (trash bag and waste bottle)

10. Cell phone

11. Clean gloves for each site

12. Proper attire for field work: eye protection, long pants, closed-toed shoes

F. SAMPLE LABELING SCHEME

Samples will be labeled according to the following scheme:

> Date (MMDDYYYY)– Sample Site – BTri – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed)

For example:

> 012320 – LCFR – BTri – R1

G. SAMPLING SITES

Sampling sites are listed in the following table. Indian Run Site 1 and Site 2 are both downstream of one of the airport's wastewater treatment facility. The site on Lytle Creek was selected downstream of the airport's second wastewater treatment facility. The site on Cowan Creek was selected upstream of both Indian Run Sites to be the control sampling site.

Sample Site Name	Coordinates	Site Description
Cowan Creek (CCJKR)	39.407615, -83.798064	Sample next to bridge on Jenkins Road crossing Cowan Creek
Indian Run Site 1 (IRJKR1)	39.411386, -83.795392	Sample after crossing field, downstream from treatment facility on Jenkins Road
Indian Run Site 2 (IRJKR2)	39.408914, -83.799194	Sample after going through wooded area next to Cowan Creek and crossing field, downstream from treatment facility on Jenkins Road
Lytle Creek (LCFR)	39.437051, -83.797386	Downstream and across the road from treatment facility, Lytle Creek right off Fife Road. Sample next to large pipe

H. SAMPLE COLLECTION PROCEDURE

1. Before going to sampling sites, clean and label sample containers and assemble sampling materials according to this protocol.
2. In the lab, calibrate the YSI Multi-meter using buffers and standards according to SOP 13.0. Remember to put an ice pack in your sample cooler.
3. When sampling the sites, stand downstream of sampling and sample into the current.
4. Upon arrival at each sampling site, put on gloves and glasses.
5. Next, collect 400 mL of site water into an amber bottle (leaving 100 mL of headroom for expansion upon freezing). Making sure the cap is on securely, place the bottle next to the ice pack in a second cooler. Repeat with second sampling bottle.
6. Use the calibrated YSI Multi-meter to measure DO, pH, specific conductance, ammonium, ammonia, and temperature of the water. Also record the ambient temperature and weather conditions. Record all readings on the Data Form.

7. Proceed to the next sampling site making sure to collect any waste. Check to be sure the GPS coordinates match. Collect all water samples and place them in the coolers. Take water quality measurements at each site. Record any additional information on the data sheet. Take photos to show conditions and anything unusual.
8. Return samples to the laboratory upon completion of sampling. Immediately place the samples into the freezer.
9. Rinse the YSI Multimeter electrodes with DI water and replace the clear plastic covers being sure that the small sponge inside has been rinsed with DI water.

I. DATA AND RECORDS MANAGEMENT

Immediately upon returning to the laboratory, be sure Standard Sampling Forms and laboratory notebooks are secured.

J. QUALITY ASSURANCE AND QUALITY CONTROL

Include a description of any replicate samples that are taken. Describe any events that may make samples invalid, spills, possible mislabeled samples, etc.

K. ATTACHMENTS

Water Data Table

Water Data Table

Date:_____

Personnel:_____

Sample Site	LCFR	IRJKR	CCJKR
Time			
Ambient Temp. (°C)			
Water Temp. (°C)			
pH			
DO (%)			
DO (mg/L)			
NH_4^+ (mg/L)			
NH_3 (mg/L)			
Conductivity (µS/cm)			
Pressure (mmHg)			
Observations			

Figure A1 Satellite view of sample sites and air park.[25]

Water Data Table

Date: 11/13/ 2019

Personnel: Lee Raska, Travis Luncan, Jessica Wiese, Clara Leedy

Sample Site	LCFR	IRJKR	CCJKR
Time	8:37	9:26	9:07
Ambient Temp. (°C)	-10	-8.89	-10
Water Temp. (°C)	4.4	2.4	0.5
pH	7.1	7.5	7.52
DO (%)	59.8	69.7	80.8
DO (mg/L)	7.62	9.59	11.65
NH_4^+ (mg/L)	1.19	0.37	0.23
NH_3 (mg/L)	0	0	0
Conductivity (µS/cm)	758	442.7	336.2
Pressure (mmHg)	742.8	743.3	743.1
Observations	Some flow; very clear	Cloudy; Muddy; Mossy	Dead deer in water; iced

Water Data Table

Date: 11/20/ 2019

Personnel: Lee Raska, Travis Luncan, Clara Leedy

Sample Site	LCFR	IRJKR	CCJKR
Time	8:13	8:47	8:33
Ambient Temp. (°C)	5	6	6
Water Temp. (°C)	8.2	6.8	5.8
pH	7.77	7.87	7.82
DO (%)	77.6	73.3	73.3
DO (mg/L)	9.17	8.9	9.16
NH_4^+ (mg/L)	0.95	0.27	0.28
NH_3 (mg/L)	0.01	0	0
Conductivity (µS/cm)	643	565	427.1
Pressure (mmHg)	7737.3	738.4	738.2
Observations	Clear water; Good flow	Deer prints; Cloudy grey water	Dead deer still present; murky water

Water Data Table

Date: 01/14/ 2020

Personnel: Lee Raska, Travis Luncan, Clara Leedy

Sample Site	LCFR	IRJKR	CCJKR
Time	10:55	9:55	10:15
Ambient Temp. (°C)	2.78	2.78	2.78
Water Temp. (°C)	7.3	6.9	6.7
pH	7.53	7.26	7.61
DO (%)	88.7	91.5	93.7
DO (mg/L)	10.7	11.06	11.44
NH_4^+ (mg/L)	0.31	0.19	0.09
NH_3 (mg/L)	0	0	0
Conductivity (µS/cm)	511	424.5	363.4
Pressure (mmHg)	736.7	737.1	737.6
Observations	Brownish murky water; tunnels	Grey blue water; Deer prints	High murky grey-blue-green water; Can't see deer

Water Data Table

Date: 01/23/ 2020

Personnel: Lee Raska, Travis Luncan, Clara Leedy

Sample Site	LCFR	IRJKR	CCJKR
Time	10:30	9:30	9:55
Ambient Temp. (°C)	-2.8	-1	-1
Water Temp. (°C)	3.2	3	2.2
pH	7.55	7.13	7.61
DO (%)	95.8	94.8	98
DO (mg/L)	12.8	12.73	13.43
NH_4^+ (mg/L)	0.29	0.19	0.08
NH_3 (mg/L)	0	0	0
Conductivity (µS/cm)	548	415.2	346.5
Pressure (mmHg)	738.3	739.2	739.1
Observations	Green moss; Raccoon prints; small bit of ice	Ice along bank; greenish slightly murky water	Some ice; no more deer

Water Data Table

Date: 02/11/ 2020

Personnel: Lee Raska, Travis Luncan

Sample Site	LCFR	IRJKR	CCJKR
Time	9:05	10:45	11:04
Ambient Temp. (°C)	0*	0*	0*
Water Temp. (°C)	5.2	5	4.8
pH	6.97	7.58	7.67
DO (%)	---**	86.4	88
DO (mg/L)	---**	10.98	11.38
NH_4^+ (mg/L)	0.66	0.24	0.12
NH_3 (mg/L)	0	0	0
Conductivity (µS/cm)	679	434.9	304.1
Pressure (mmHg)	734.4	735.3	735.3
Observations	High water; good flow; Raccoon prints	Greenish water; new site: can see confluence; run out banks	Brownish water; Good flow

*Ambient temperature looked up after sampling

**Errors occurred with YSI Multimeter Pro Plus

Water Data Table

Date: 02/25/ 2020

Personnel: Lee Raska, Travis Luncan, Clara Leedy

Sample Site	LCFR	IRJKR	CCJKR
Time	10:12	9:55	9:40
Ambient Temp. (°C)	5*	5*	5*
Water Temp. (°C)	5.4	5	5.5
pH	7.36	7.09	6.92
DO (%)	92.4	92.7	96.3
DO (mg/L)	11.69	11.82	12.15
NH_4^+ (mg/L)	0.32	0.2	0.22
NH_3 (mg/L)	0	0	0
Conductivity (µS/cm)	299.4	276.1	264.7
Pressure (mmHg)	726.4	726.8	726.8
Observations	Slightly green and turbid; moderate flow; T.L. Turbidity 27”	Very muddy; high flow; T.L. Turbidity 9”	Muddy; High flow; Small patch of foam; T.L. Turbidity 4”

*Ambient temperature looked up after sampling

Water Data Table

Date: 03/10/ 2020

Personnel: Lee Raska, Travis Luncan, Clara Leedy

Sample Site	LCFR	IRJKR	CCJKR
Time	9:45	9:08	9:28
Ambient Temp. (°C)	12.2	12.2	12.2
Water Temp. (°C)	10.6	9.8	9.6
pH	7.85	6.94	7.43
DO (%)	89.1	77.3	88
DO (mg/L)	9.91	8	10.01
NH_4^+ (mg/L)	0.46	0.48	0.14
NH_3 (mg/L)	0.01	0	0
Conductivity (µS/cm)	232.3	342.8	388.8
Pressure (mmHg)	733.4	733.9	733.8
Observations	High Flow; T.L Turbidity 8”	Moderate Flow; Grey/green water T.L. Turbidity 8”	Clear; Moderate Flow; T.L. Turbidity 34”

Rained throughout sampling and was darkly overcast

Table A1 Water Data Table Averages

Parameter	LCFR	IRJKR	CCJKR
Ambient Temperature (°C)	1.5225	2.01125	1.8725
Water Temperature (°C)	5.5375	4.8625	4.3875
DO (%)	71.91429	73.2125	77.2625
DO (mg/L)	8.841429	9.135	9.9025
pH	6.51625	6.42125	6.5725
NH_4^+ (mg/L)	0.5225	0.2425	0.145
NH_3 (mg/L)	0.0025	0	0
Conductivity(uS/cm)	458.8375	362.65	303.85
Specific Conductance (uS/cm)	724.3375	581.2375	506.8
Pressure (mmHg)	643.6625	644.25	644.2375

APPENDIX B

Standard Operating Procedure

DETERMINATION OF BENZOTRIAZOLE AND ANALOG COMPOUNDS BY LIQUID CHROMATOGRAPHY – MASS SPECTROMETRY IN SURFACE AND GROUND WATER SAMPLES

October 8, 2019

Audrey McGowin, PhD Jessica Wiese

A. SCOPE AND APPLICATION

1H-benzotriazoles are complexing agents that are widely used as anti-corrosives, engine coolants, aircraft de-icers, anti-freezing liquids, and silver protection in dishwashing agents. Chemically, 1H-benzotriazoles are soluble in water, resistant to biodegradation, only partially removed in wastewater treatment, and have the potential to pass drinking water treatment. Most benzotriazole (BTri) compounds and their analogs are polar and thermally labile. In addition, BTris are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTri and BTri analogs are deposited. The procedures outlined in this SOP were created for the qualitative and quantitative determination of BTri and similar compounds by Liquid Chromatography – Mass Spectrometry (LC-MS) in surface and ground water samples.

B. SUMMARY OF METHOD

The purpose of this Standard Operating Procedure (SOP) is to establish a procedure for the qualitative and quantitative determination of 1H-benzotriazoles, tolytriazoles, and comparable analogs using LC-MS instrumentation.

C. HEALTH AND SAFETY

The analyst must assume that all surface water samples are potentially contaminated and should be treated accordingly. Personal protection equipment (PPE) should be worn at all times while in the lab; this includes lab coat, nitrile gloves, safety glasses, long pants and closed-toe shoes. Material safety data sheets (MSDS) can be found in the back left corner of the lab. Organic solvents should be handled cautiously and used in a fume hood.

D. SAFETY AND CAUTIONS

1. All personnel must abide by the safety procedures discussed in the “Wright State University Chemical Hygiene Plan.” Any spills or emergency accidents must be reported to the department of Environmental Health and Safety at Wright State University for assistance.

2. Material safety data sheets for all chemical reagents are available and should be read and understood by all personnel performing the methods described herein.

3. All personnel must wear a lab coat, gloves, and appropriate eye protection when in the laboratory, including visitors.

4. Containers and boxes must be labeled with the chemical, the date, its concentration and hazard, the expiration date, and the name of the personnel responsible.

5. During instrument operation, personnel must wear protective gloves and safety glasses.

E. EQUIPMENT AND SUPPLIES

1. Agilent Technologies 1220 Infinity LC quadrupole LCMS system that includes the following components:

a. Agilent Eclipse Plus C18 (1.8 µm I.D 2.1 x 100 mm) column

b. Autosampler

c. Agilent 1220 Infinity LC variable wavelength detector (VWD)

d. OpenLAB CDS ChemStation Software

e. Single quadrupole mass analyzer

2. 2-mL autosampler vials with Teflon caps.

3. Various glassware (Pasteur pipettes, volumetric flasks, amber jars/vials) for standard solution and eluent solution preparation.

4. Type 3 fixed needle syringes (100-µL, 250- µL, and 500- µL)

5. Chemicals & Reagents

a. HPLC-grade Methanol (MeOH, CAS #67-56-1)

b. Water (Milli-Q purified)

c. Formic Acid (CAS #64-18-6)

d. 1H-benzotriazole (BTri, CAS # 95-14-7)

f. 4-methyl-1H-benzotriazole (4-Me-BTri, CAS #249-921-1)

g. 5-methyl-1H-benzotriazole (5-Me-BTri, CAS #136-85-6)

h. 5,6-dimethyl-1H-benzotriazole (5,6-dimethyl-BTri, CAS #4184-79-6)

F. PROCEDURE – ELUENT SOLUTION PREPARATION

1. Add 1.0 mL of formic acid to 1 L of MeOH and mix thoroughly.

2. Add 1.0 mL of formic acid to 1 L of water and mix thoroughly.

3. Transfer each solution to a 1-L glass bottle and hook each bottle up to the LC-MS.

G. PROCEDURE – STANDARD SOLUTION PREPARATION

1. Weigh out 0.00500 g of BTri and dissolve it in 50.0 mL MeOH to create the 100- ppm standard solution.

2. Take 2.5 mL of the 100 ppm solution and dilute to 25.0 mL with MeOH to create the 10-ppm standard solution.

3. Take 250 µL of the 100 ppm solution and dilute to 25.0 mL with MeOH to create the 1.0-ppm standard solution.

4. Take 250 µL of the 10 ppm solution and dilute to 25.0 mL with MeOH to create the 100-ppb standard solution.

5. Take 1.25 mL of the 1.0 ppm solution and dilute to 25.0 mL with MeOH to create the 50-ppb standard solution.

6. Take 250 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 25-ppb standard solution.

7. Take 100 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 10-ppb standard solution.

8. Repeat steps 1-7 for both 4-Me-BTri and 5-Me-BTri. 9. Store all standard solutions in amber glass vials/jars at -20 ℃.

H. PROCEDURE – SURROGATE STANDARD SOLUTION PREPARATION

1. Weigh out 0.00025 g of 5,6-dimethyl-BTri and dissolve it in 50.0 mL of MeOH to create the 5.0-ppm standard solution.

2. Take 5.00 mL of the 5.0 ppm solution and dilute to 25.0 mL with MeOH to create the 1.0-ppm standard solution.

3. Take 1.00 mL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 100-ppb standard solution.

4. Take 500 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 50-ppb standard solution.

5. Take 250 µL of the 1.0 ppm solution and dilute to 10.0 mL with MeOH to create the 25-ppb standard solution.

6. Take 1.00 mL of the 100 ppb solution and dilute to 10.0 mL with MeOH to create the 10-ppb standard solution.

7. Store all standard solutions in amber glass vials/jars at -20 ℃.

I. PROCEDURE – LC-MS ANALYSIS

1. Make sure the nitrogen tank is full. If empty, contact Dr. McGowin to replace as soon as possible. If the tank is not running already, open the two black valves on the pressure valve, and the grey valve on the tank over the "gas use" label; the pressure should read around 500 – 600 kPa.

2. If the LC-MS has not been used in a while, it is important to check that it is tuned properly.66 3. Go to “MSD Tune” and click “ATUNES TUN”.

4. Select positive or negative polarity.

5. Under “Tune”, click “Check Tune”.

6. The system will run a tune check and automatically generate a report that says whether it is a “Pass” or “Fail”.

7. If it passes, proceed to Step 3; if it fails, go to “Calibrate” and run a calibration test. Make sure to save the new calibration results.

8. Run an “Autotune” check under positive, negative, or dual polarity. If it passes; proceed to Step 3; if it fails, contact Joseph Solch or Garrett Vanness for assistance.

9. If you have a method already, skip this step. If you do not, go to the "Method" tab and click "New Method".

10. In the "Sampler" section of the "Method and Run Control" window, right click and select "Method".

11. Adjust injection volume and stop time as desired; do not change the auxiliary settings.

12. Right click the "Grad. Pump" section of the "Method and Run Control" window and click "Method" to display the following parameters to be adjusted: Flow, Solvents, Stop time and Pressure Limits.

    a. The flow should not exceed more than 1-1.5 mL/min - anything greater than that will increase the pressure on the column to such an extent that it will be permanently damaged.

    b. Under the solvents tab, enter the name of the solvent as well as the percentage of each.

    c. The stop time can be adjusted to elute the last peak you desire.

    d. You must be very mindful of the pressure limits set. Do not increase the upper pressure limit to greater than 370 bar. If a long run time is planned or you are running on low volumes of eluent, the lower pressure limit can be increased to ~ 50 bar.

13. Right click the “Column” section of the “Method and Run Control” window and click “Method”. Adjust the column temperature as desired.

14. Right click the “MSD Signals” section of the “Method and Run Control” window and click “Method” to display the following parameters to be adjusted: Polarity, Full Scan and SIM.

    a. Select positive or negative polarity as desired.

    b. It is recommended that you run your method in “Full Scan” mode for your first standard solution in order to determine the times the analyte peaks of interest elute.

    c. Once you have determined your analyte’s elution time(s), you can run in “SIM” mode.

15. Right click the “UV Lamp” section of the “Method and Run Control” window and click “Method”. Adjust the wavelength detection as desired.

16. Once your method is complete, go to the "Method" tab, click "Save Method As..." and name your method to the following code: Initials – MMDDYYYY - Primary Eluent name – MS ion mode.

17. Now that you have a method saved, you can load it for future analyses: go to the "Method" tab and click "Load Method..."; at the top of the screen you should see your method file name.

18. Turn both the LC and MS components of the system on. To do this, click the green "ON" buttons on the screen. This will start the pumping of eluent through the column.

19. You must then purge the system in order to eliminate gas bubbles from the eluent solution.

20. Go to the "Grad. Pump" section in the "Method and Run Control" window and increase the flow rate to 5.00 mL/min. You should see that the clear tube that goes to waste be degassed. Do NOT click “OK” yet.

21. Unhinge the door to the LC component, and give the black waste knob a quarter turn counterclockwise. This switches the flow of all incoming eluent to waste.

22. Click “OK”. Turn the black knob clockwise and back a few times until no more bubbles are pumped through the eluent solution.

23. Change the flow rate back according to your sample method. Turn the black knob clockwise until it is closed and put the cover of the LC component back on. Allow the pressure to stabilize (about 10-20 minutes).

24. Set up your sequence by going to the "Sequence" tab and clicking "New Sequence Template". This creates a template to which you can save new sequences as in the future.

    a. To modify your sequence, go to the "Sequence” tab and select "Sequence Table...". This will open a spreadsheet – like window.

    b. Enter the sequence of your samples, denoting the vial position (Vial), name (Method Name) and number of injections per vial (Inj/Vial).

    c. To add lines for more samples, click "Insert". To remove sample lines, click "Cut". Exit the sequence table by clicking "OK".

    d. Go to the "Sequence" tab, click "Save Sequence Template As...", and give your file a name according to the sequence file code: Initials_Date samples were taken (MMDDYYYY)_Samples Analysis

25. To run all of the samples in your sequence, click “Start Sequence”. If you want to run only one or a few of the samples in your sequence, go to the “Sequence” tab and click

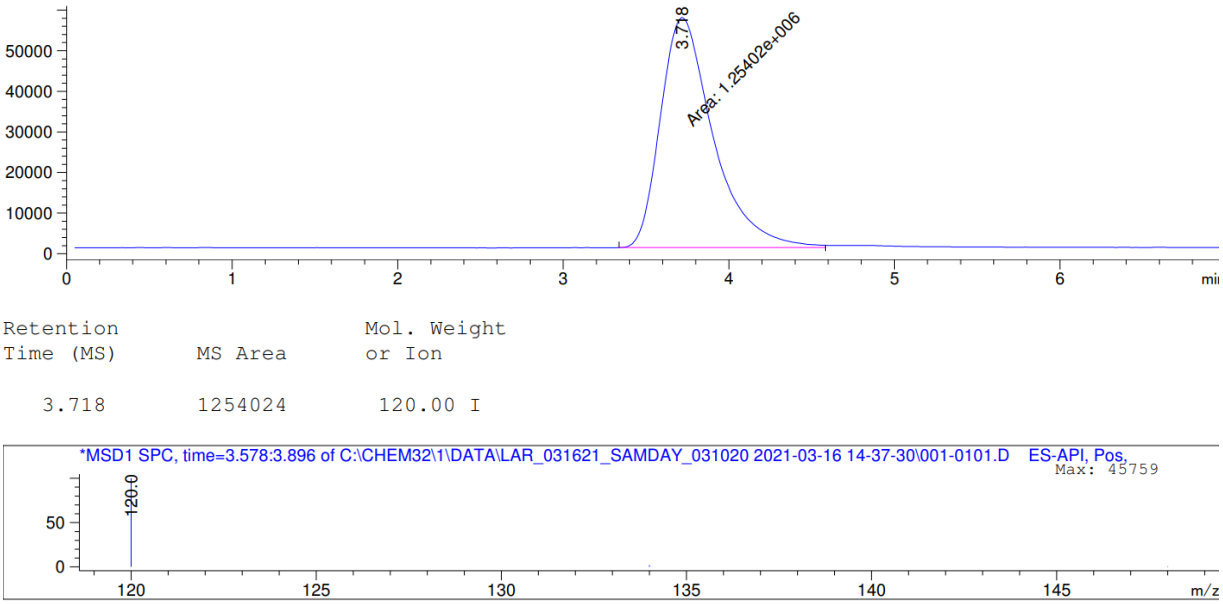
“Partial Sequence” then “New”. This allows you to then pick and choose which vials you want to run.

26. To view the data, go to the "Data Analysis" window.

27. The “Spectrum” button displays the spectra with all of the elution times of the analytes.

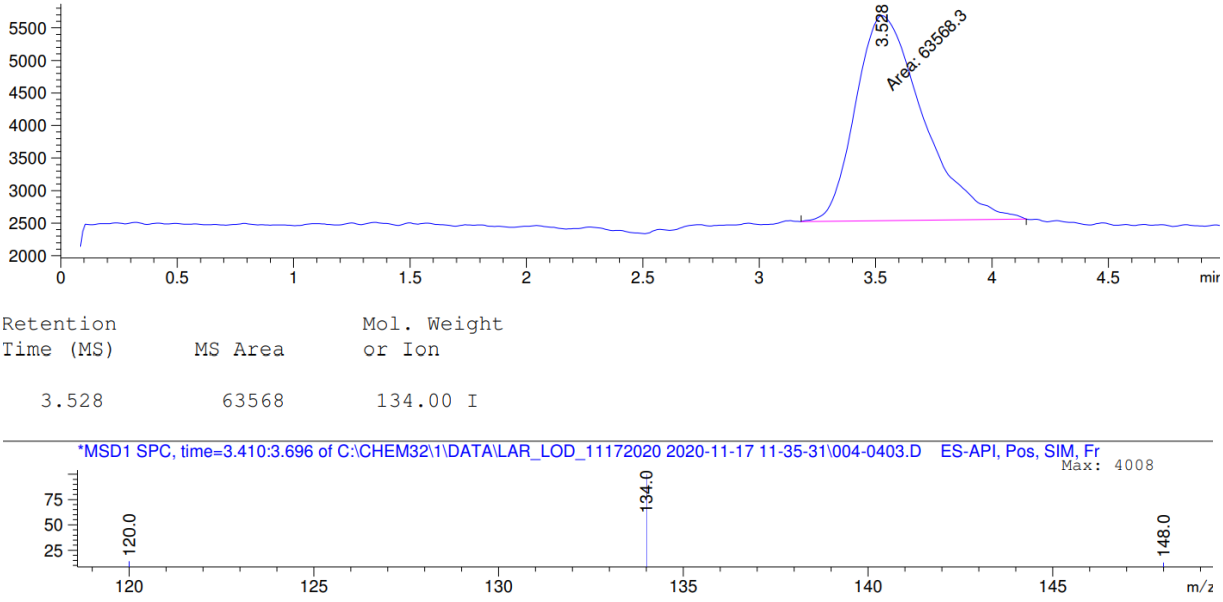
28. The “Signal” button allows you to integrate the peaks and determine the areas of each peak.

29. The “Print Report” button will display a report in the "Data Analysis" window that you can view before printing. Click the "Print" button, and this will open the PDF24 Assistant. Click "Save as PDF", and save the file as your sequence name to a USB flash drive by clicking "Save".



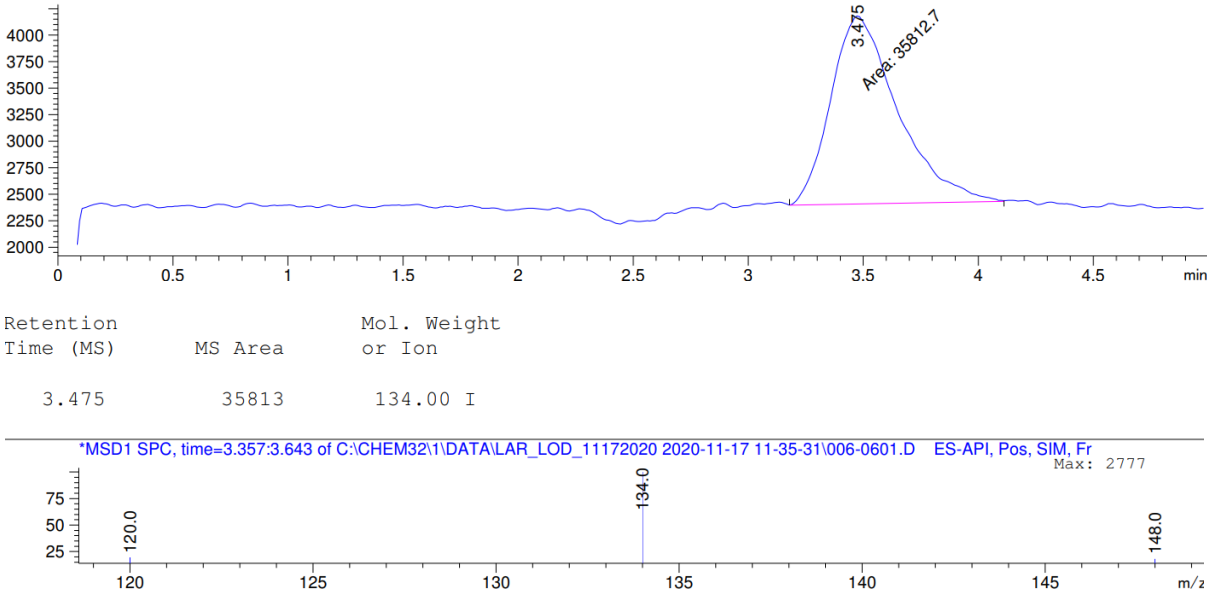
Retention Time (MS)	MS Area	Mol. Weight or Ion
3.718	1254024	120.00 I

Figure A2 Chromatogram and mass spectrum for 1002 µg/L BTZ



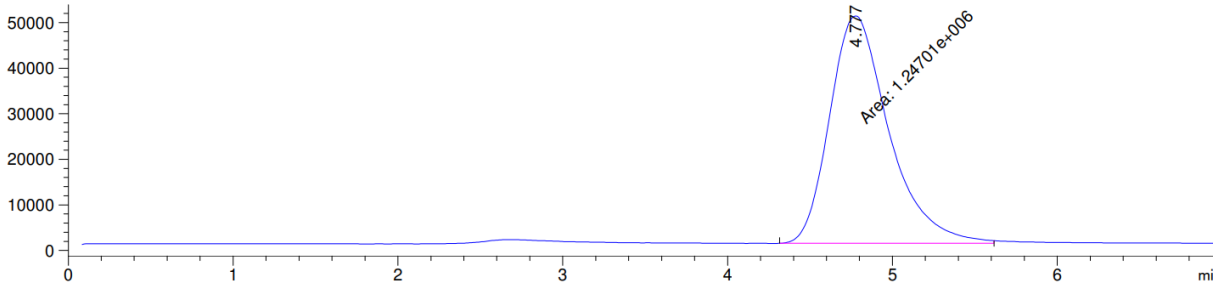
Retention Time (MS)	MS Area	Mol. Weight or Ion
3.528	63568	134.00 I

Figure A3 Chromatogram and mass spectrum for 25.05 µg/L 4-MBTZ



Retention Time (MS)	MS Area	Mol. Weight or Ion
3.475	35813	134.00 I

Figure A4 Chromatogram and mass spectrum for 25.05 µg/L 5-MBTZ



Retention Time (MS)	MS Area	Mol. Weight or Ion
4.777	1247009	134.00 I

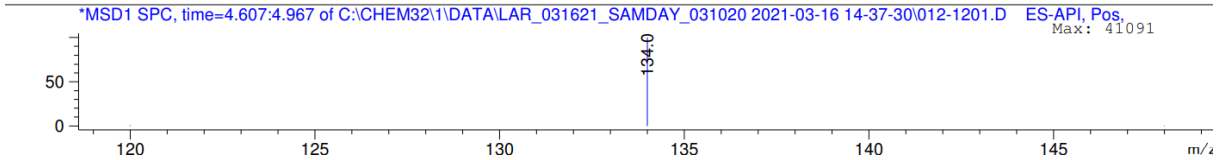


Figure A5 Chromatogram and mass spectrum for 1002 µg/L TTZ

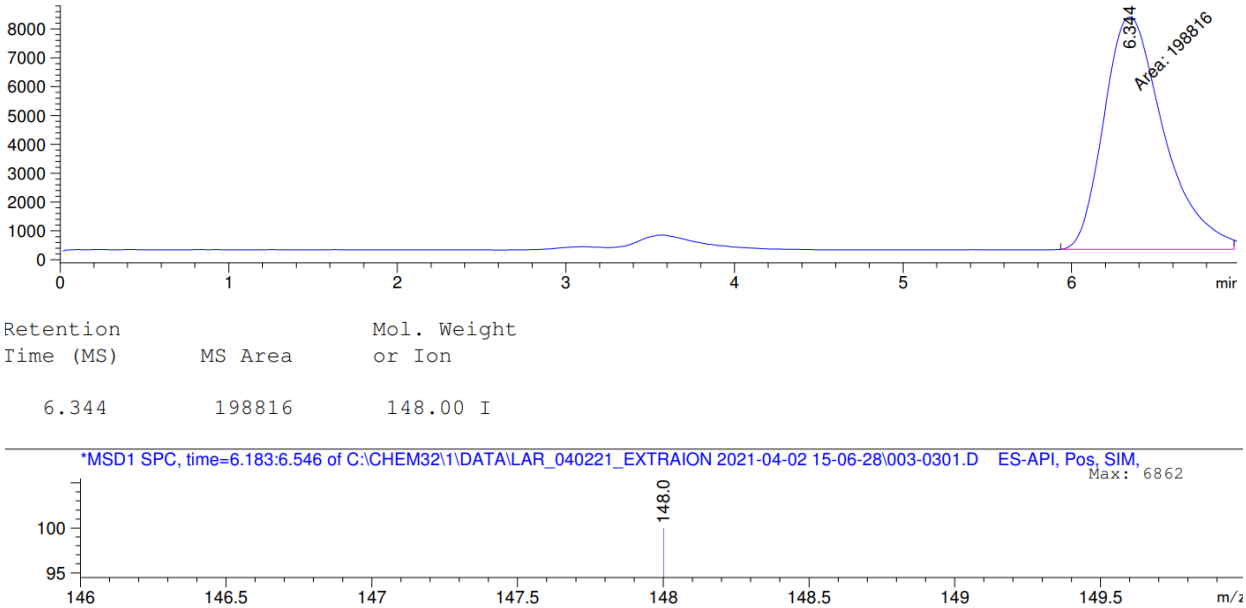


Figure A6 Chromatogram and mass spectrum for 100.2 µg/L 5,6-DMBTZ.

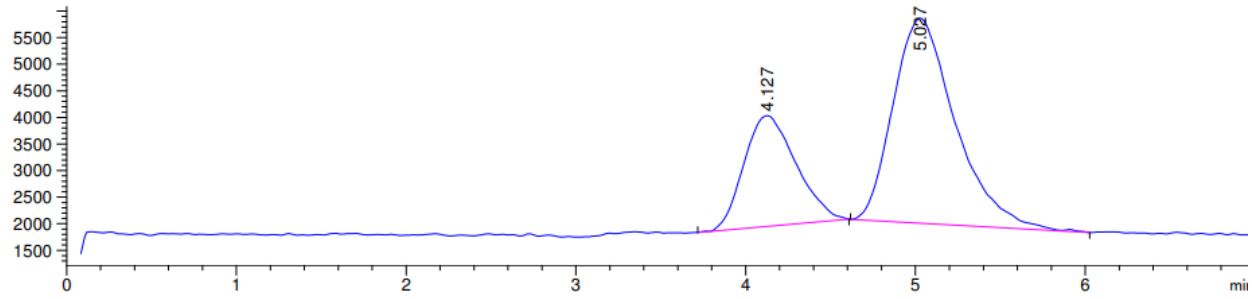


Figure A7 BTZ/TTZ mixed standard, 40:60, 0.100 µL/minute.

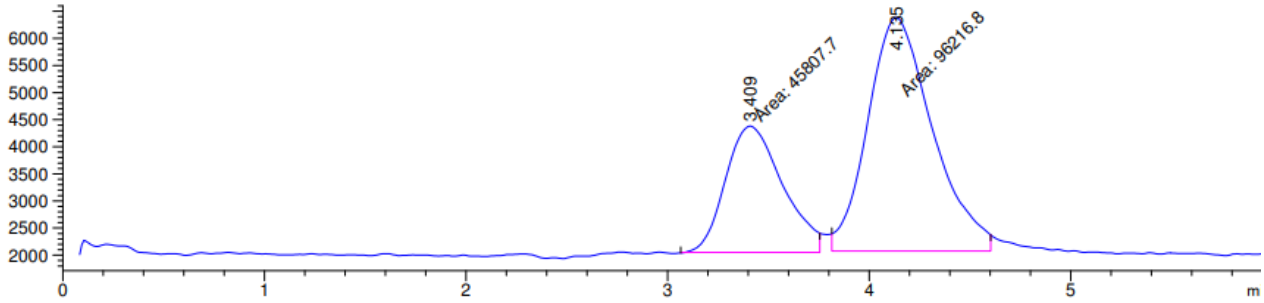


Figure A8 BTZ/TTZ mixed standard, 40:60, 0.120 µL/minute.

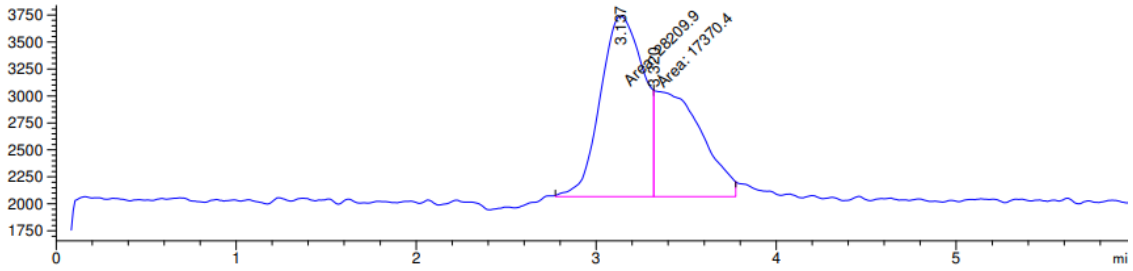


Figure A9 BTZ/TTZ mixed standard, 30:70 eluent mix, 0.120 µL/minute.

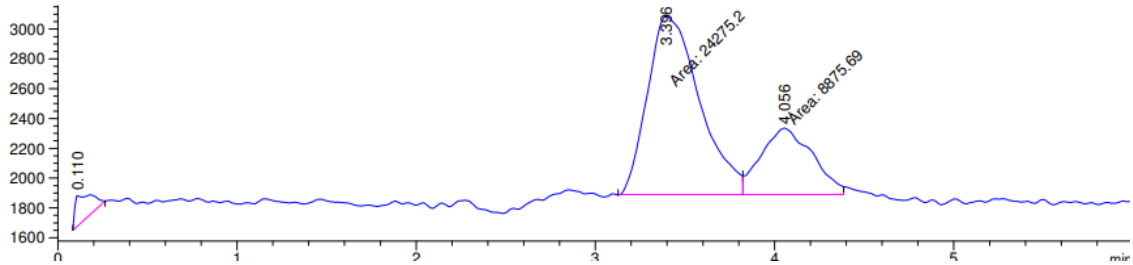


Figure A10 BTZ/TTZ mixed standard, 40:60 eluent mix, 0.120 µL/minute.

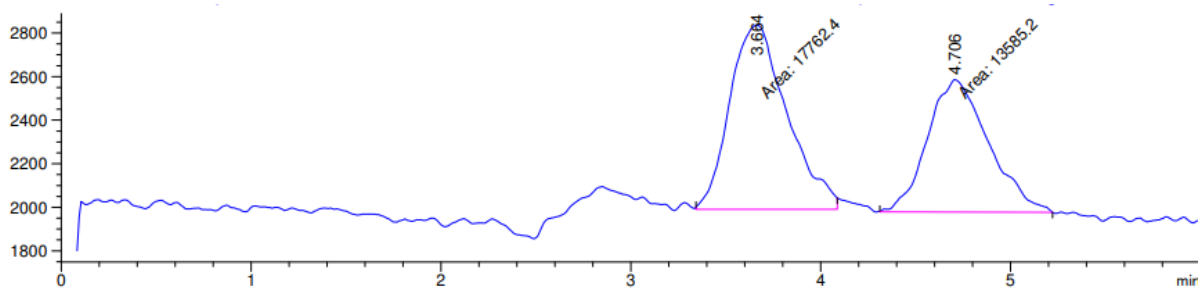


Figure A11 BTZ/TTZ mixed standard, 45:55 eluent mix, 0.120 µL/minute.

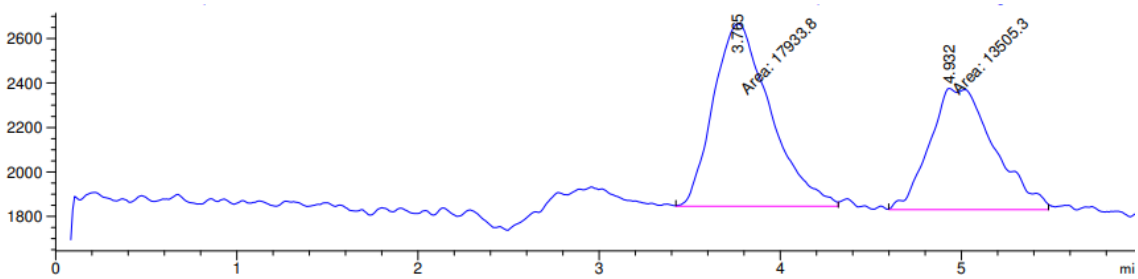


Figure A12 BTZ/TTZ mixed standard, 47:53 eluent mix, 0.120 µL/minute.

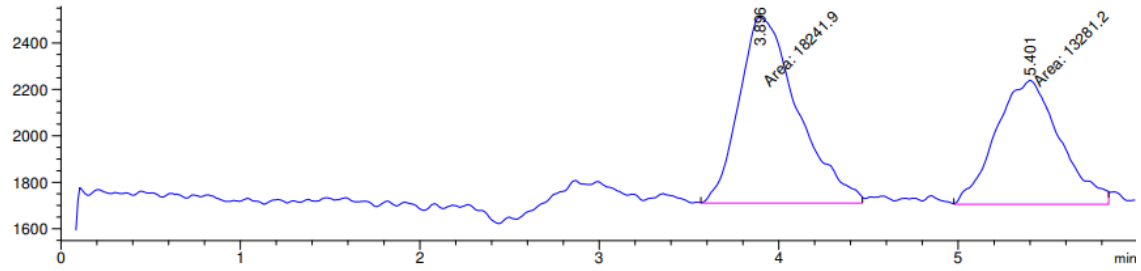


Figure A13 49:51 eluent mix, 0.120 µL/minute.

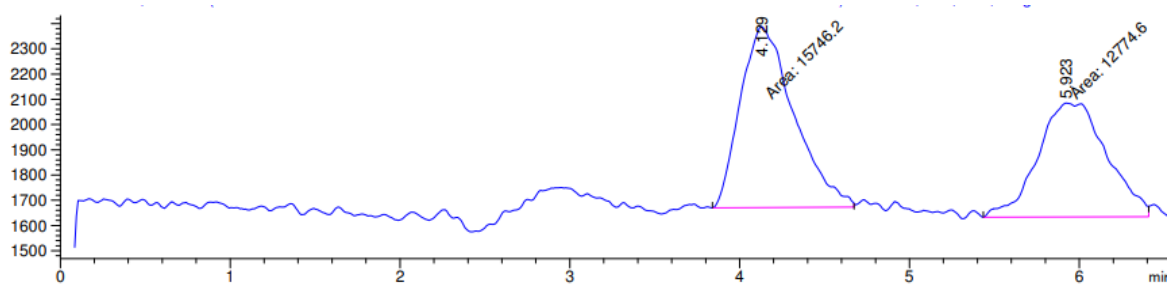


Figure A14 BTZ/TTZ mixed standard, 50:50 eluent mix, 0.120 µL/minute.

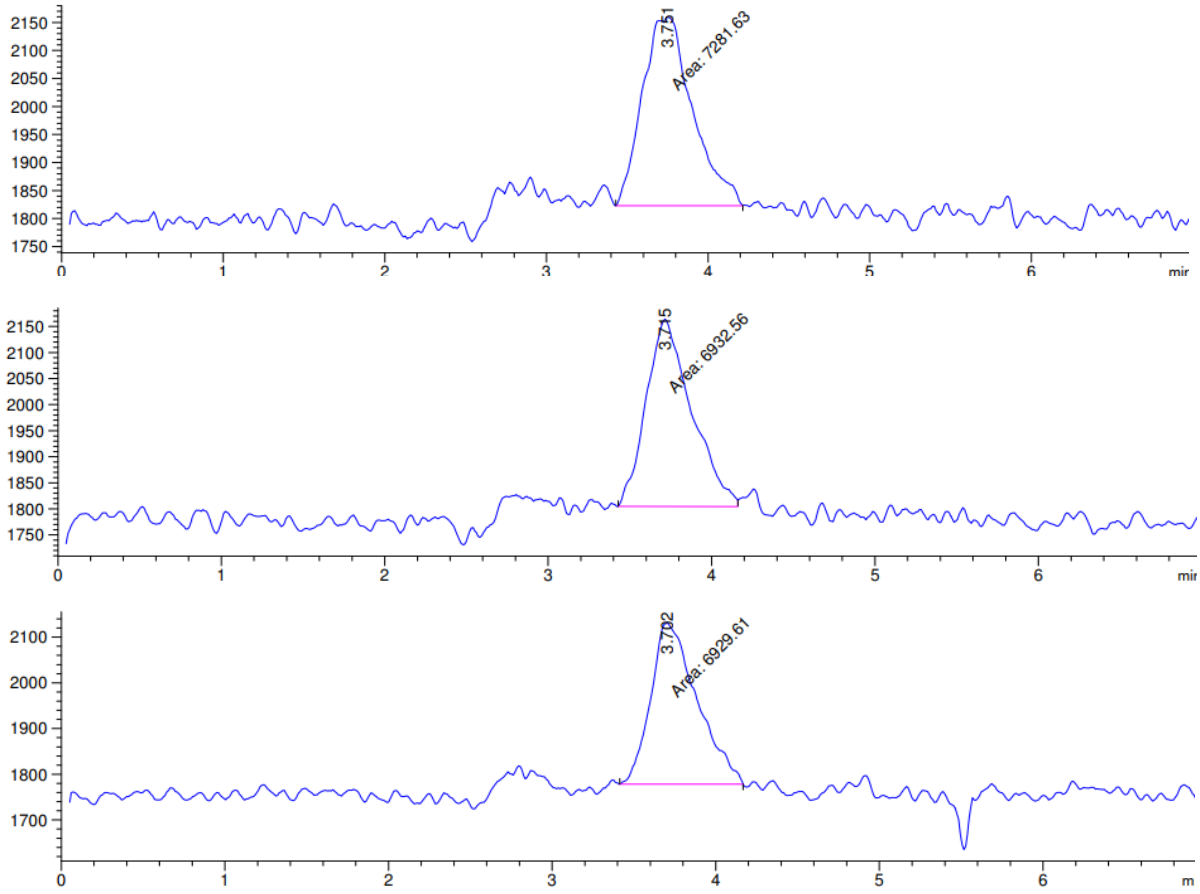


Figure A15 Three BTZ (5.01 µg/L) injections with a 45:55 eluent mix at 0.120 mL/minute.

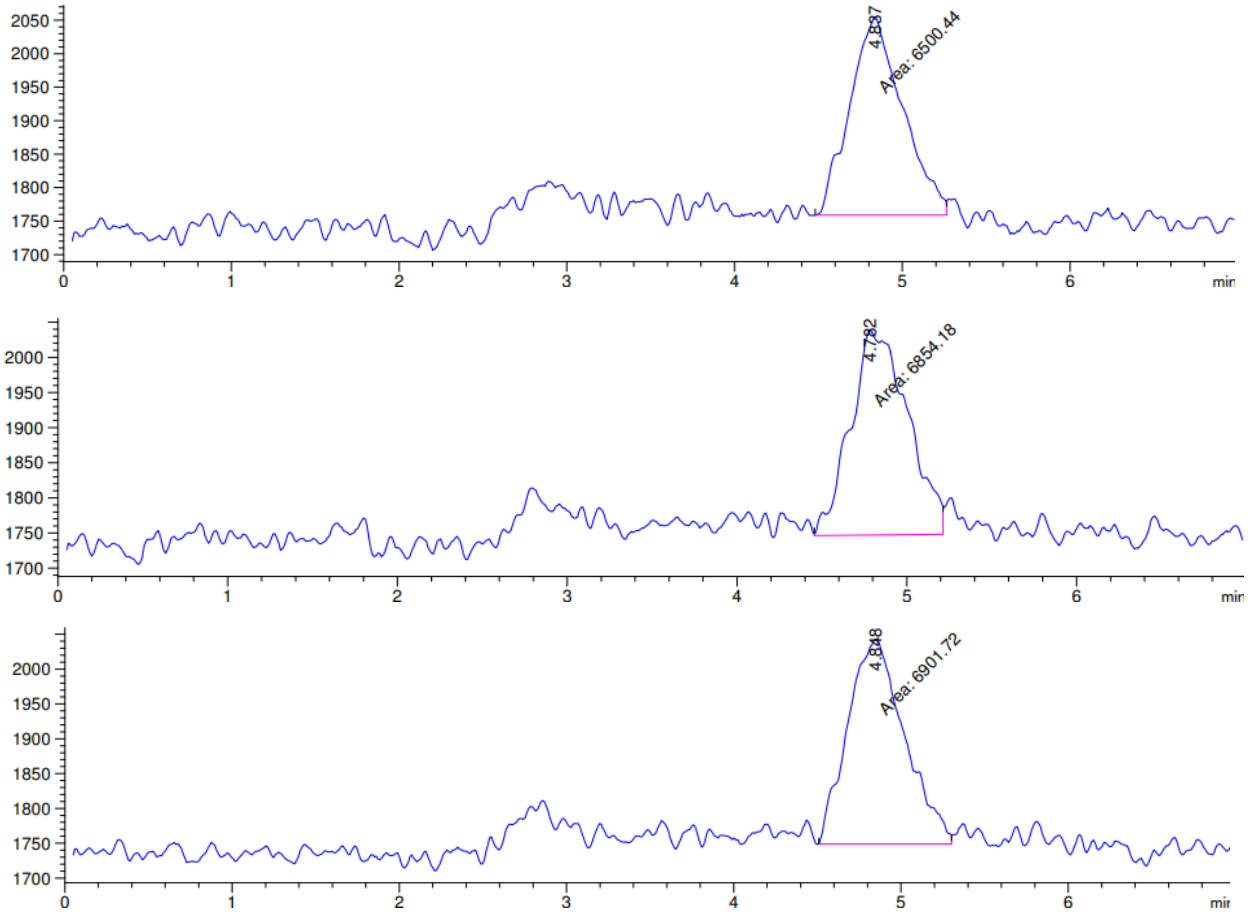


Figure A16 Three TTZ (5.01 µg/L) injections with a 45:55 eluent mix at 0.120 mL/minute.

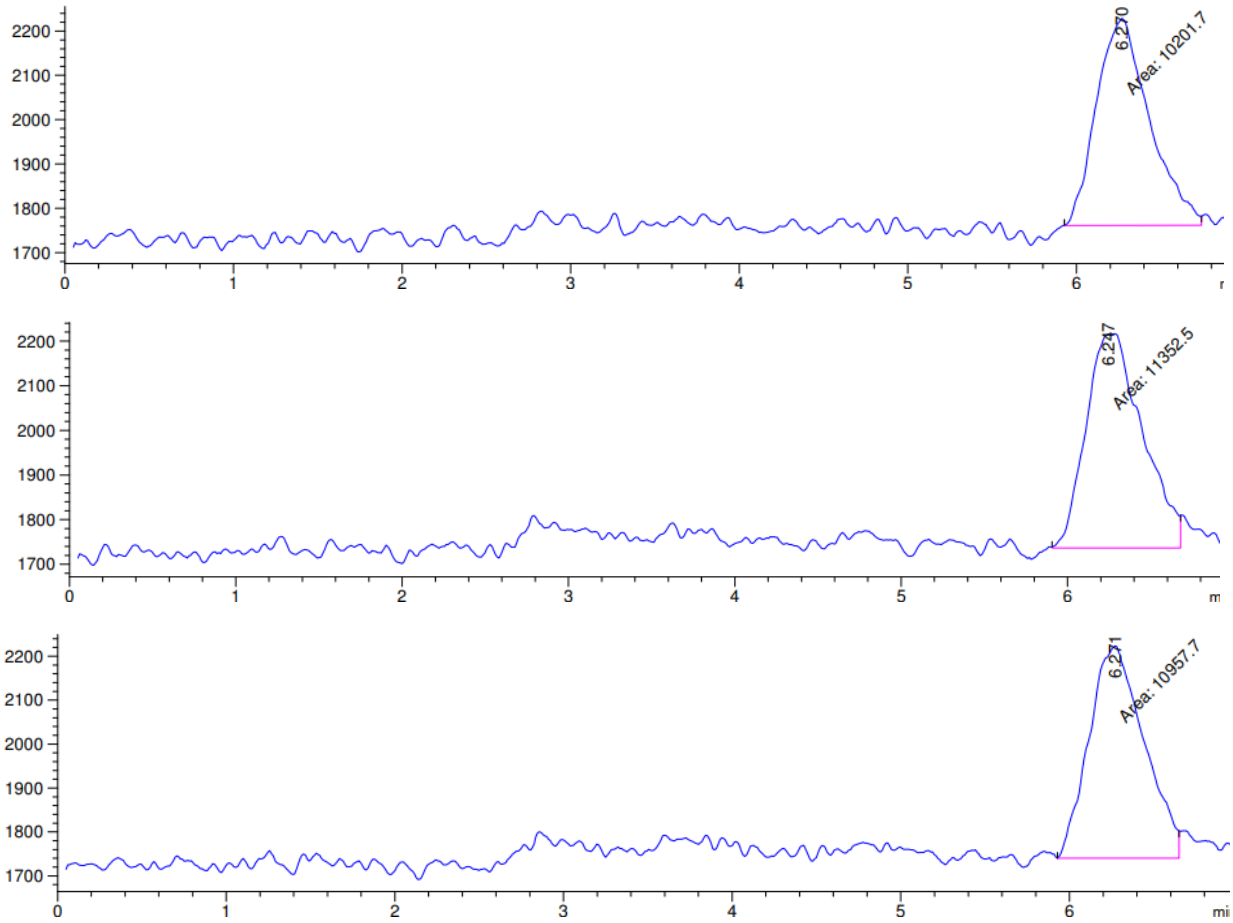


Figure A17 Three 5,6DMBTZ (5.01 µg/L) injections with a 45:55 eluent mix at 0.120 mL/minute.

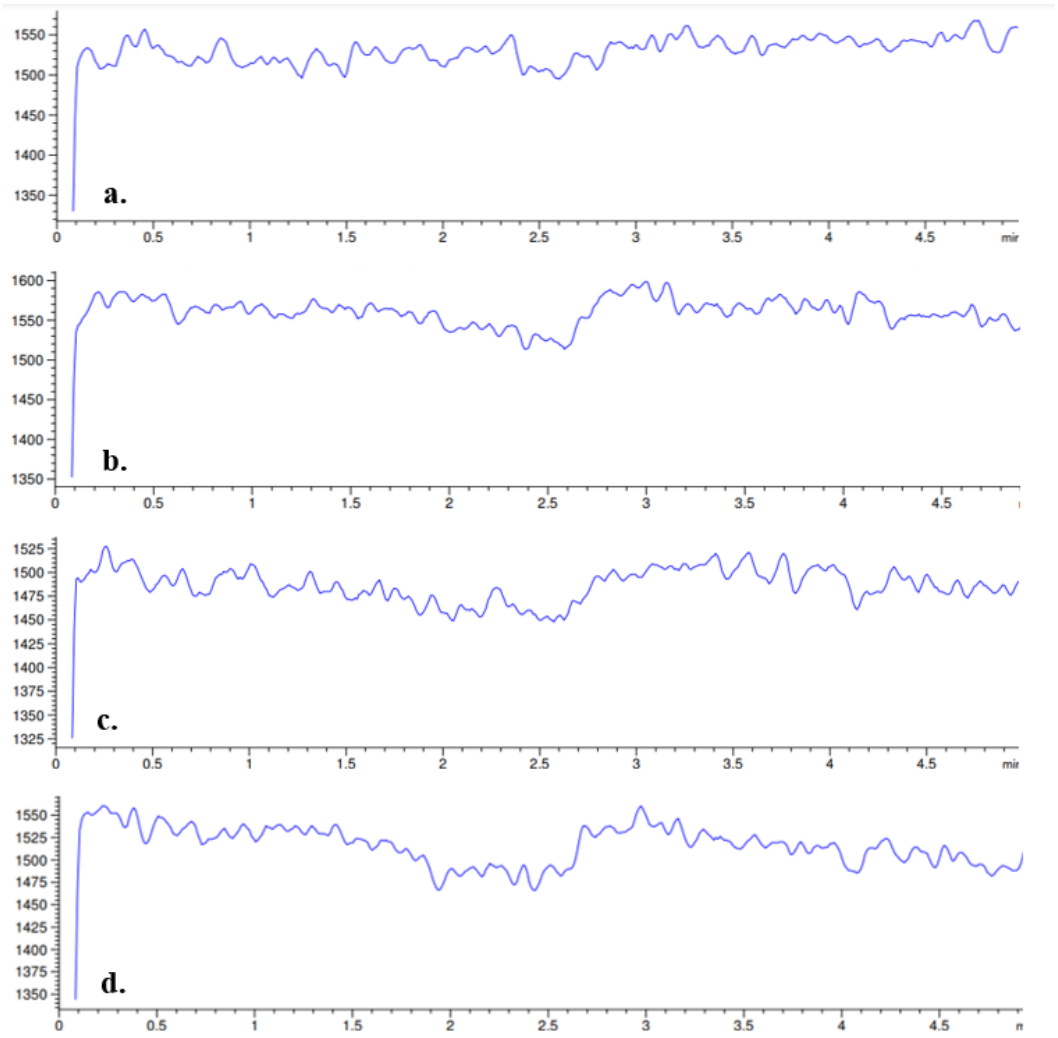


Figure A18 Methanols investigated as a potential source of contaminant peaks: a. different lab b. bench top c. MeOH opened 0208221 d. MeOH opened 011221.

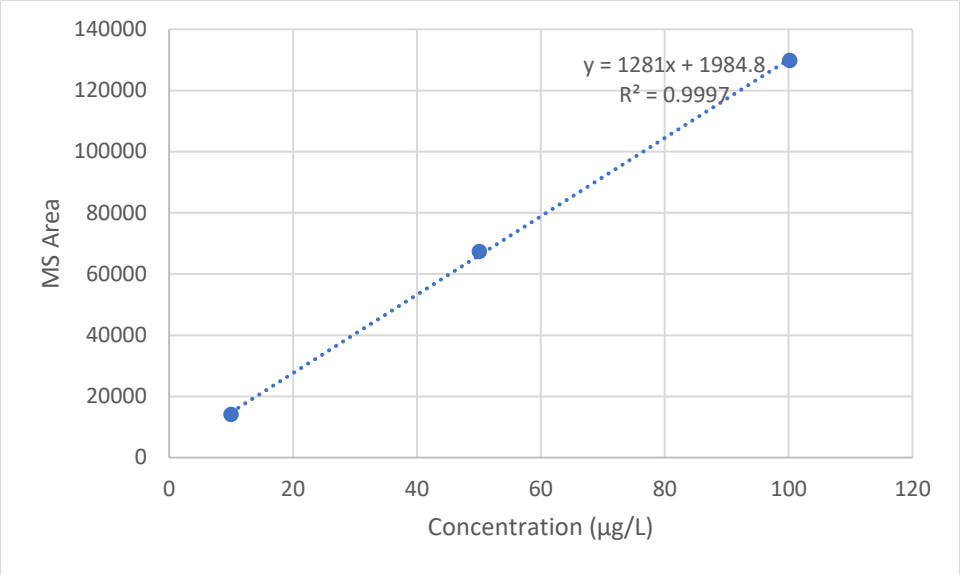


Figure A19 BTZ calibration curve for mock sample set 1 01/04/2021

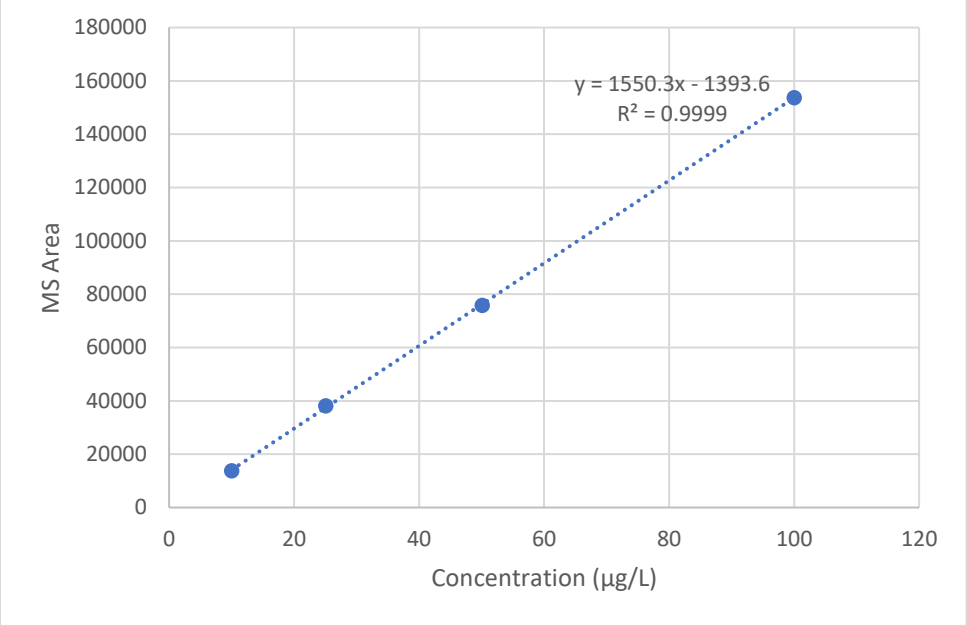


Figure A20 TTZ calibration curve for mock sample set 1 01/04/2021

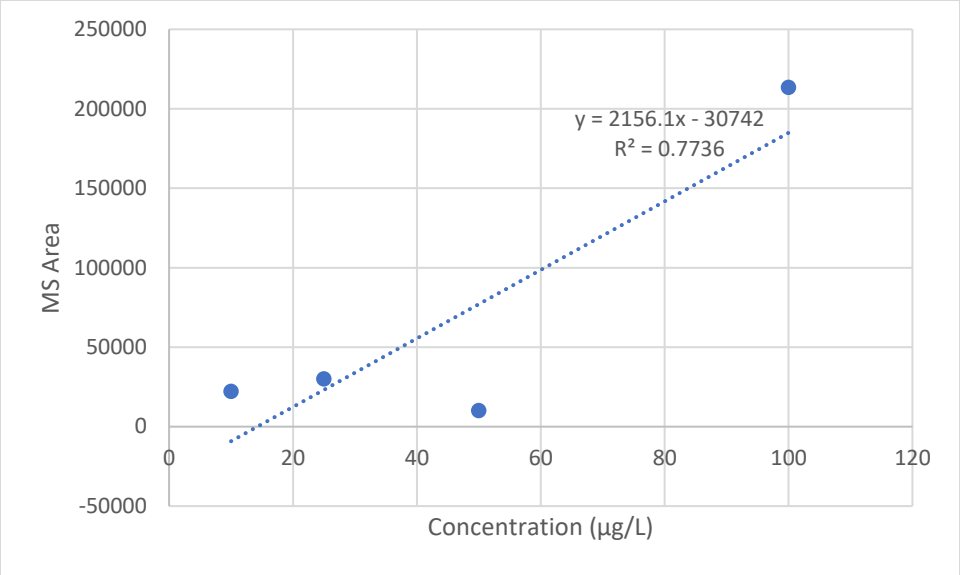


Figure A21 5,6DMBTZ calibration curve for mock sample set 1 01/04/2021

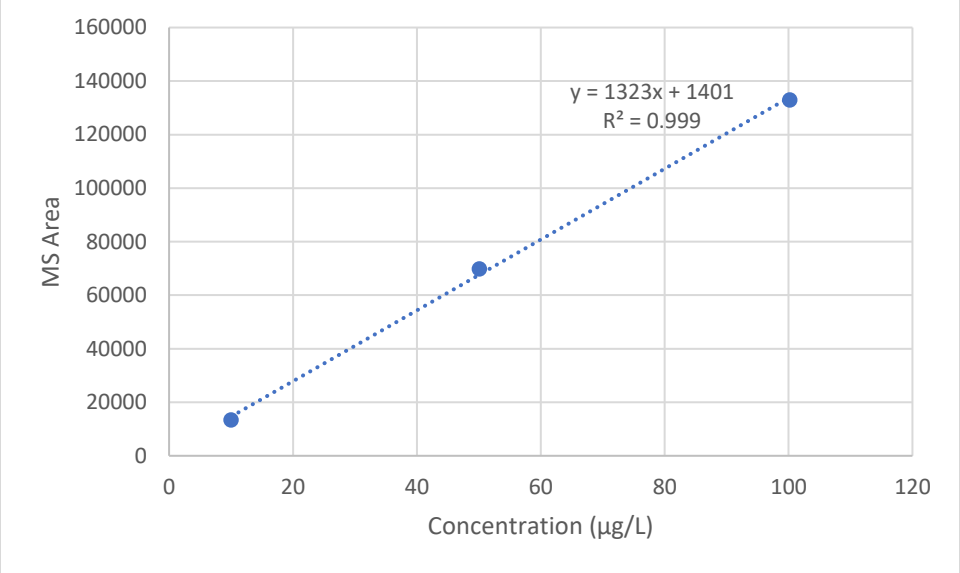


Figure A22 BTZ calibration curve for mock sample set 2 01/15/2021

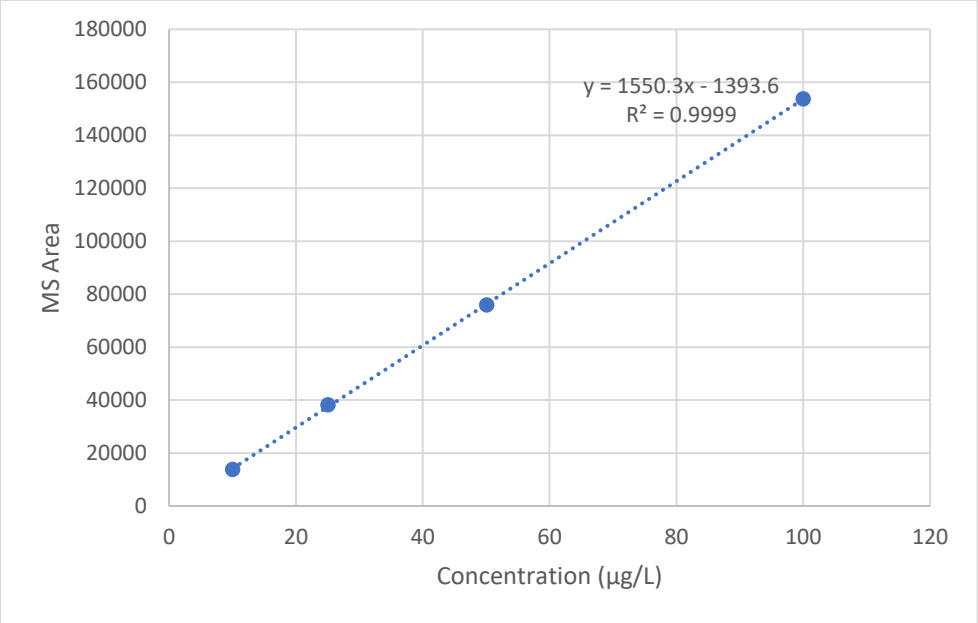


Figure A23 TTZ calibration curve for mock sample set 2 01/15/2021

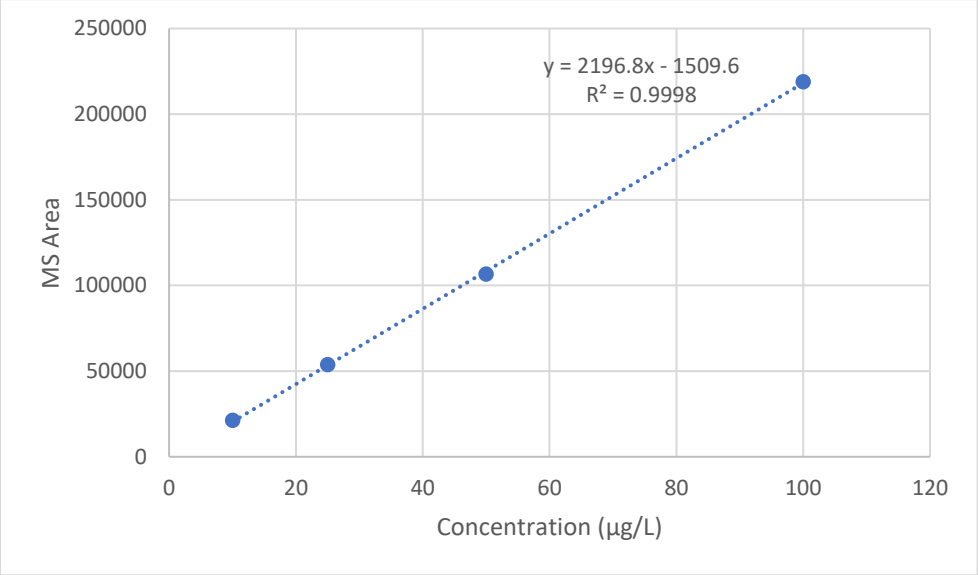


Figure A24 5,6DMBTZ calibration curve for mock sample set 2 01/15/2021

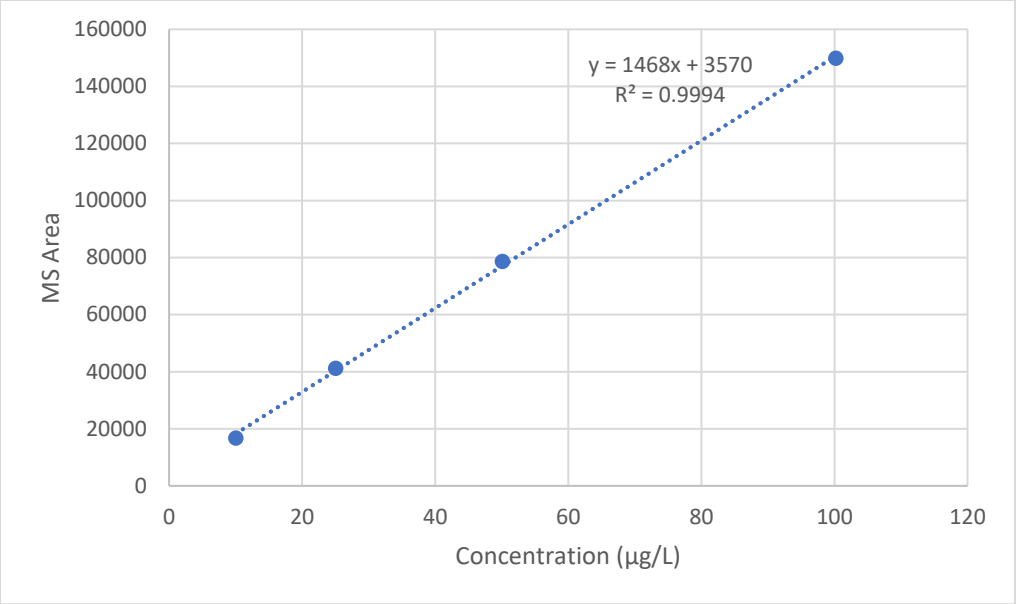


Figure A25 BTZ calibration curve for 12/04/2019 samples.

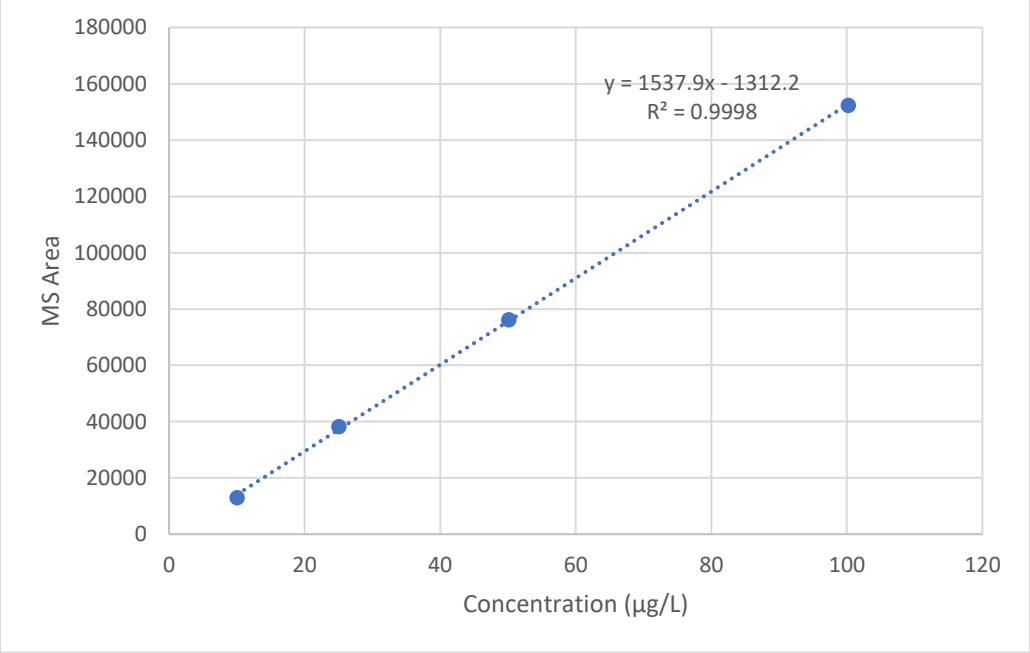


Figure A26 TTZ calibration curve for 12/04/2019 samples.

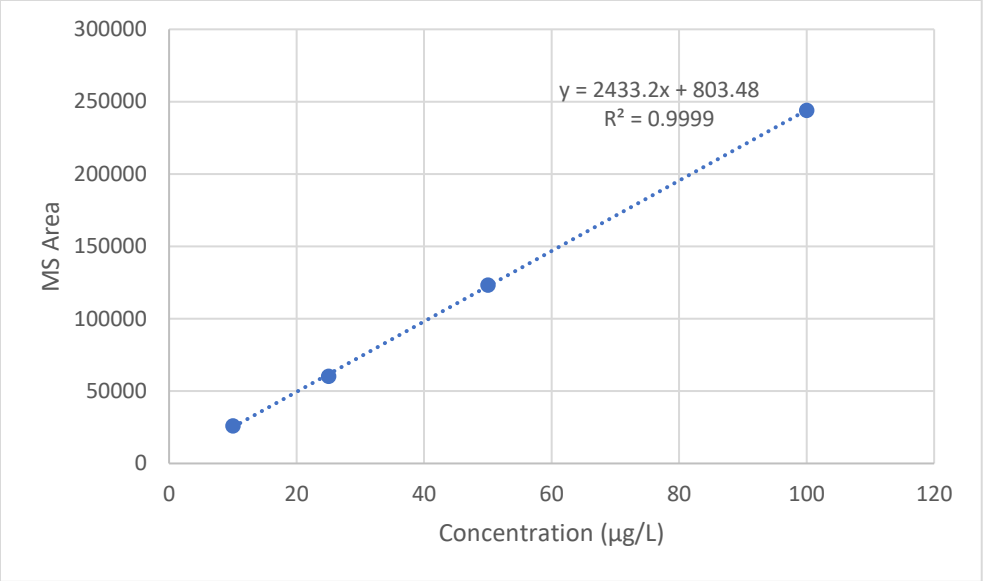


Figure A27 5,6DMBTZ calibration curve for 12/04/2019 samples.

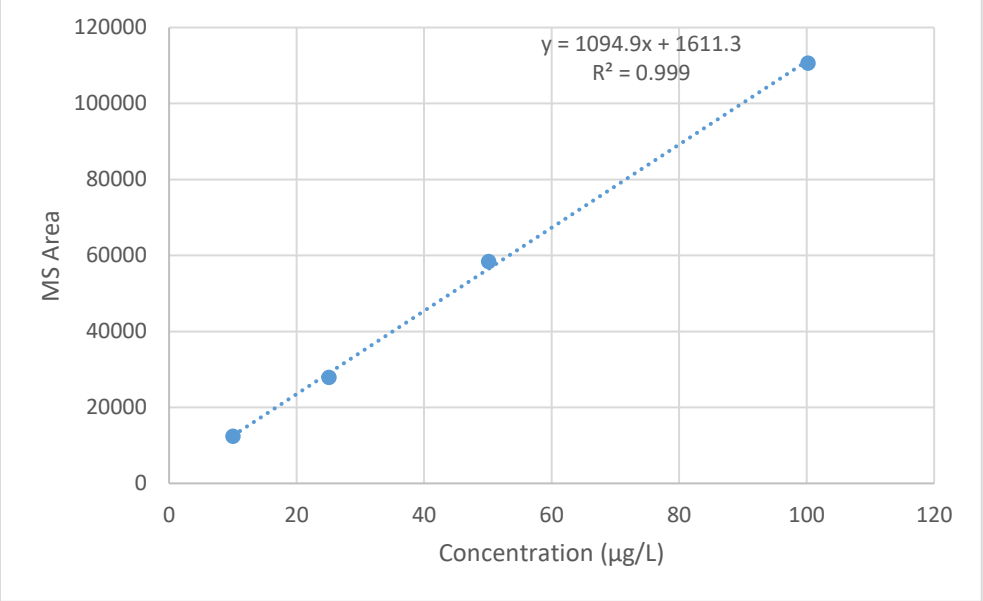


Figure A28 BTZ calibration curve for 11/13/2019 samples.

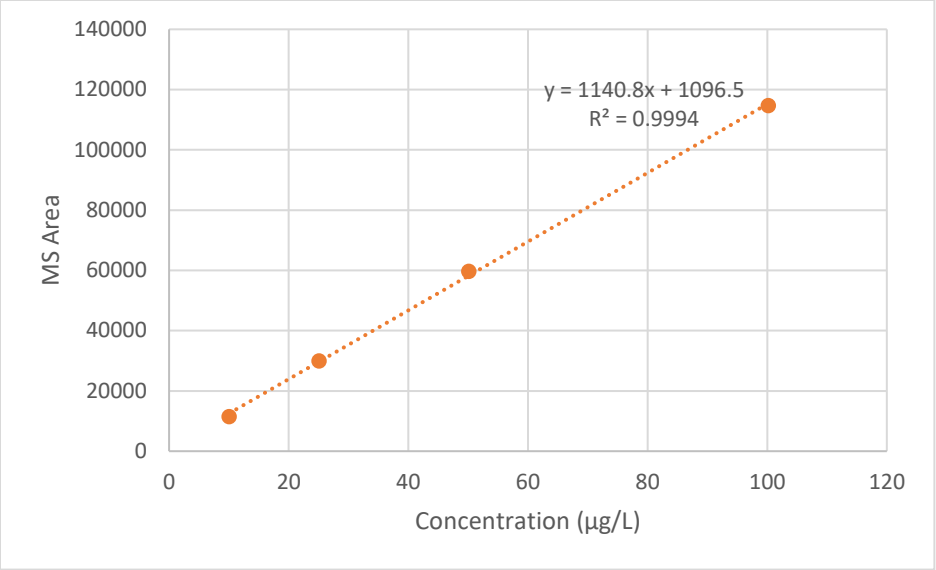


Figure A29 TTZ calibration curve for 11/13/2019 samples.

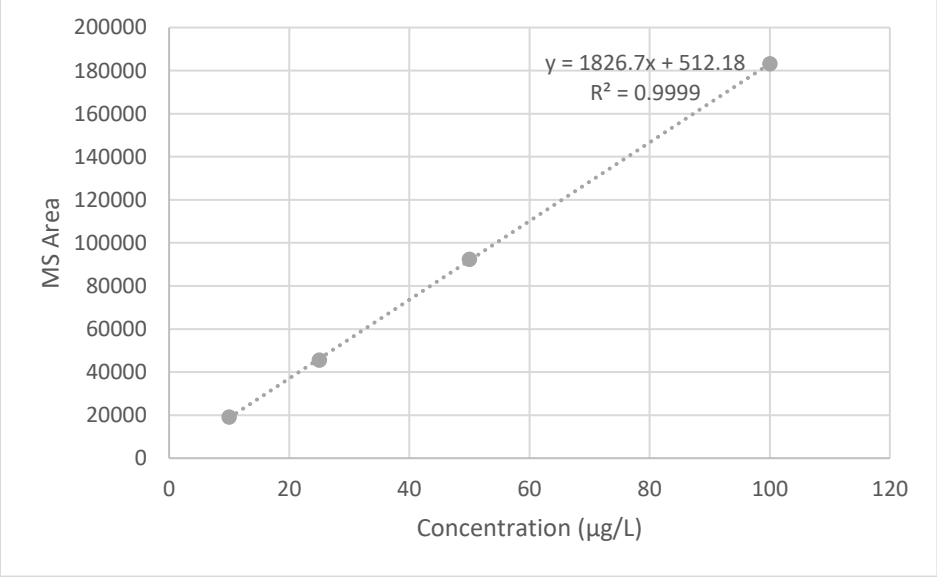


Figure A30 5,6DMBTZ calibration curve for 11/13/2019 samples.

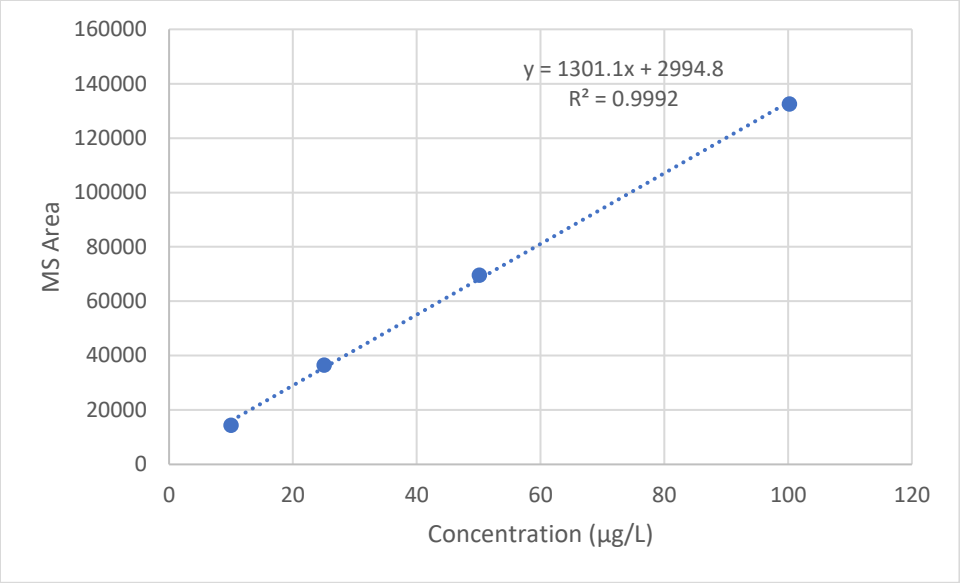


Figure A31 BTZ calibration curve for 11/20/2019 samples.

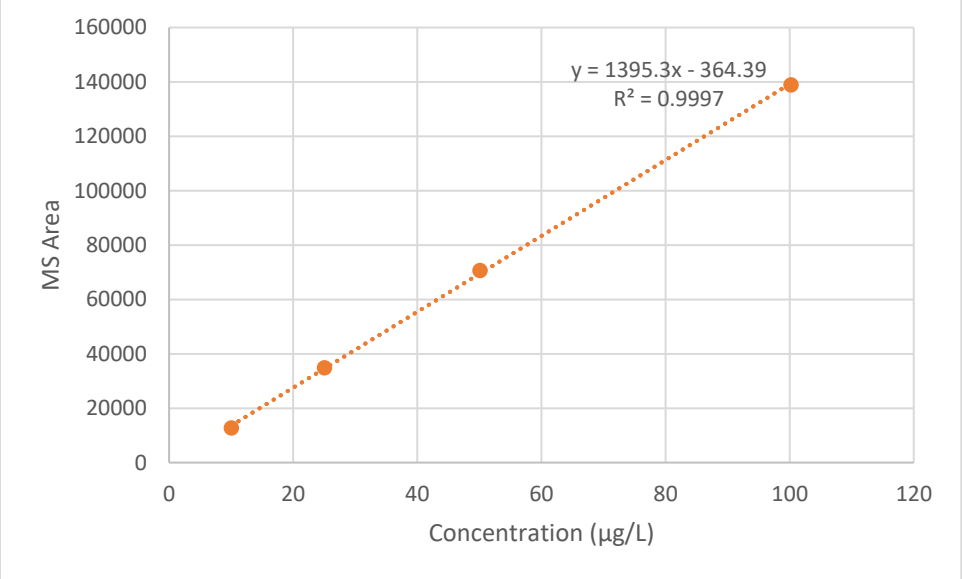


Figure A32 TTZ calibration curve for 11/20/2019 samples.

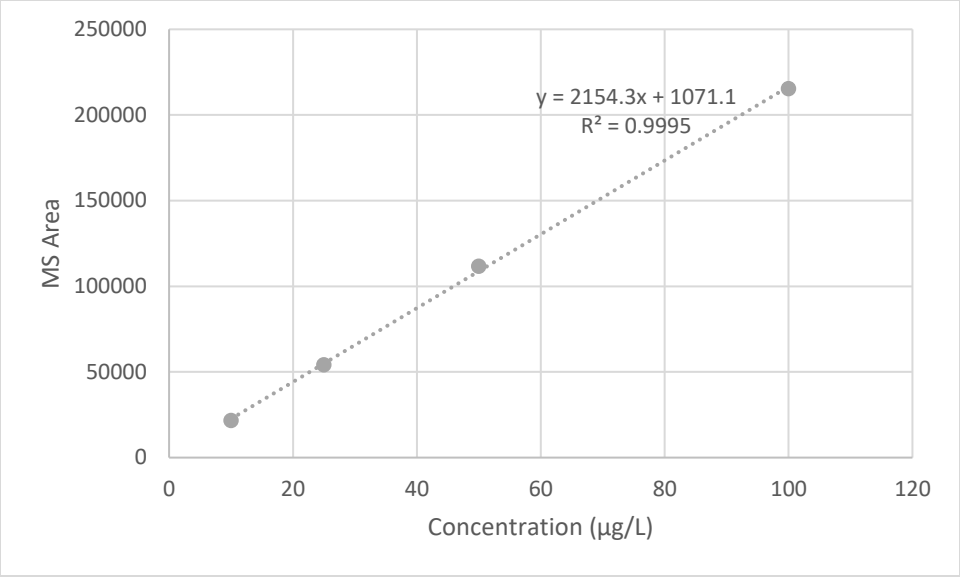


Figure A33 5,6DMBTZ calibration curve for 11/20/2019 samples.

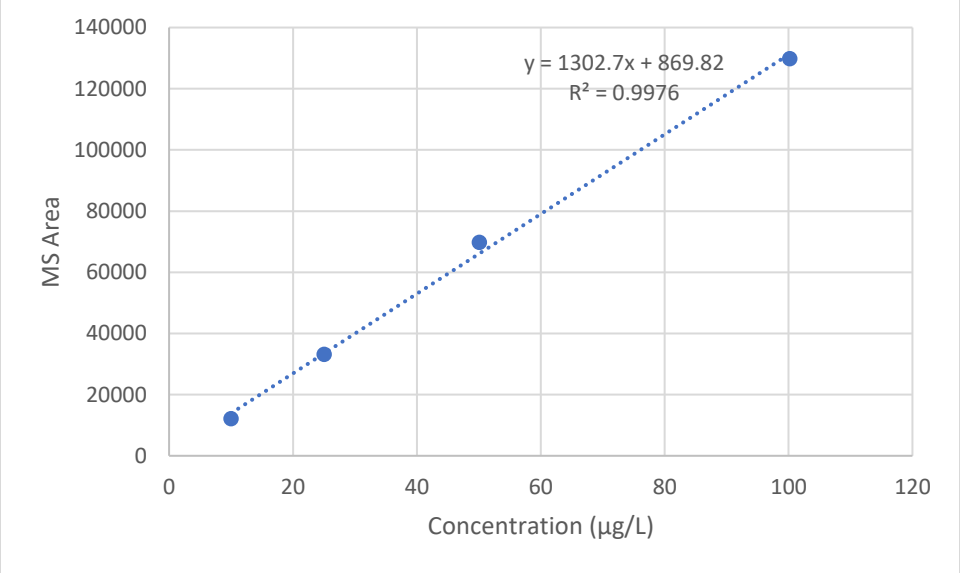


Figure A34 BTZ calibration curve for 01/14/2020 samples.

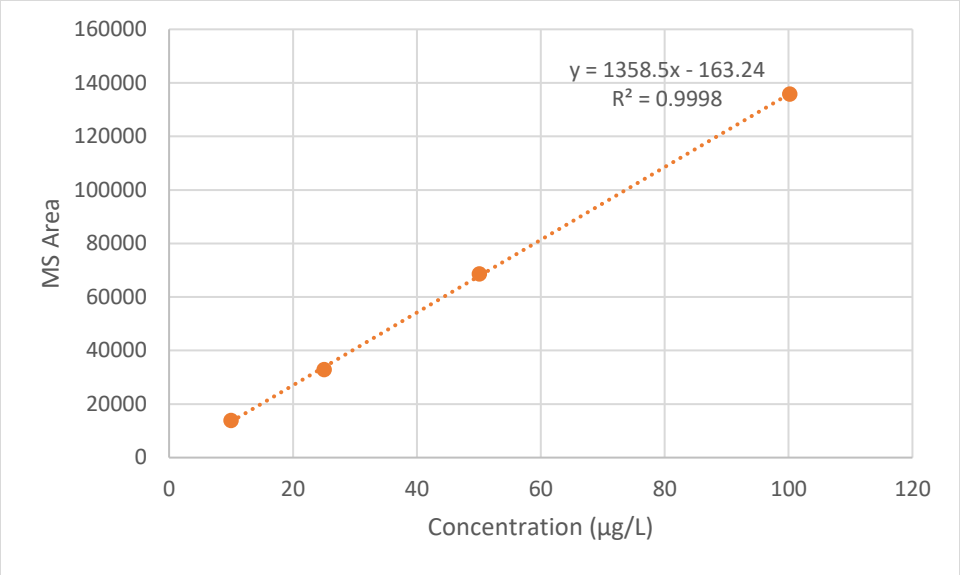


Figure A35 TTZ calibration curve for 01/14/2020 samples.

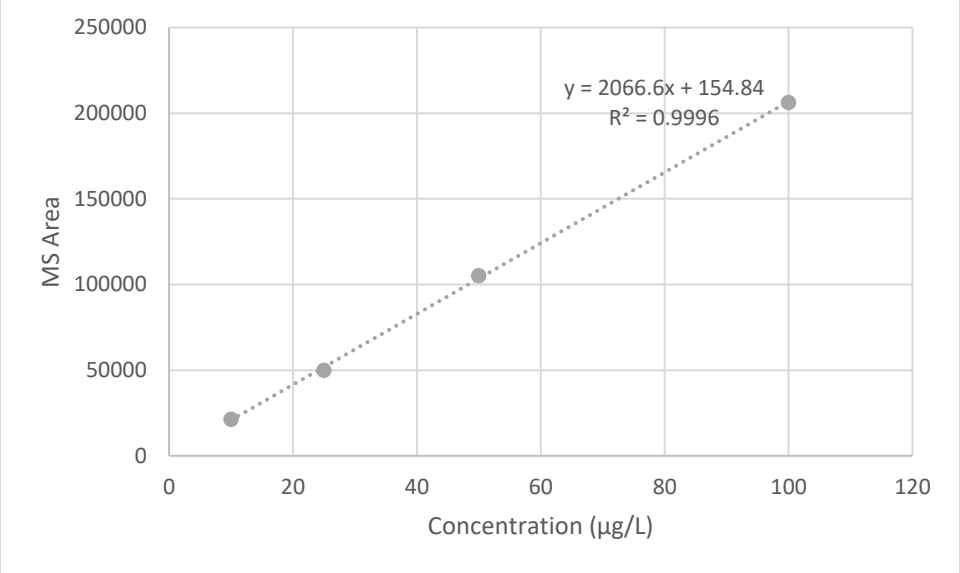


Figure A36 5,6DMBTZ calibration curve for 01/14/2020 samples.

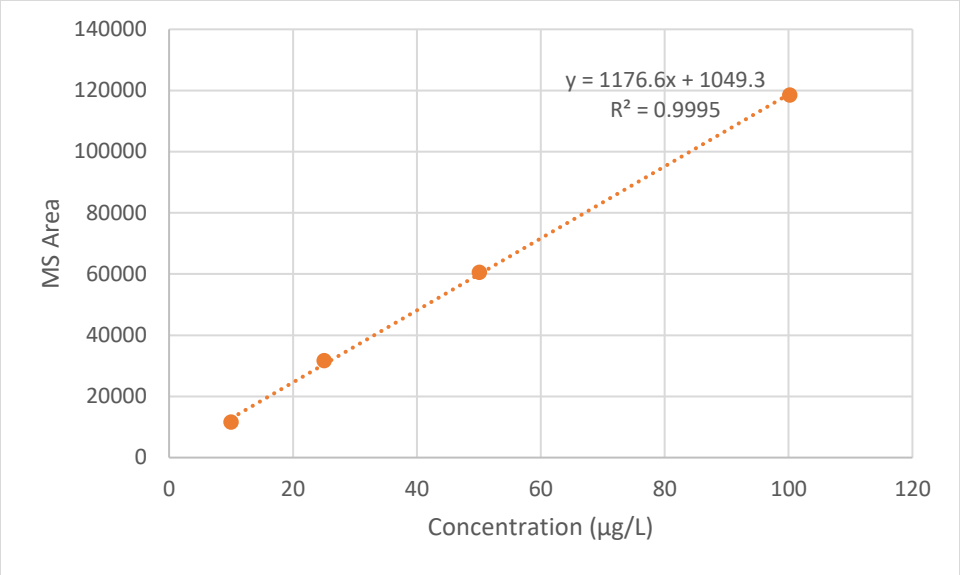


Figure A37 TTZ calibration curve for 01/23/2020 samples.

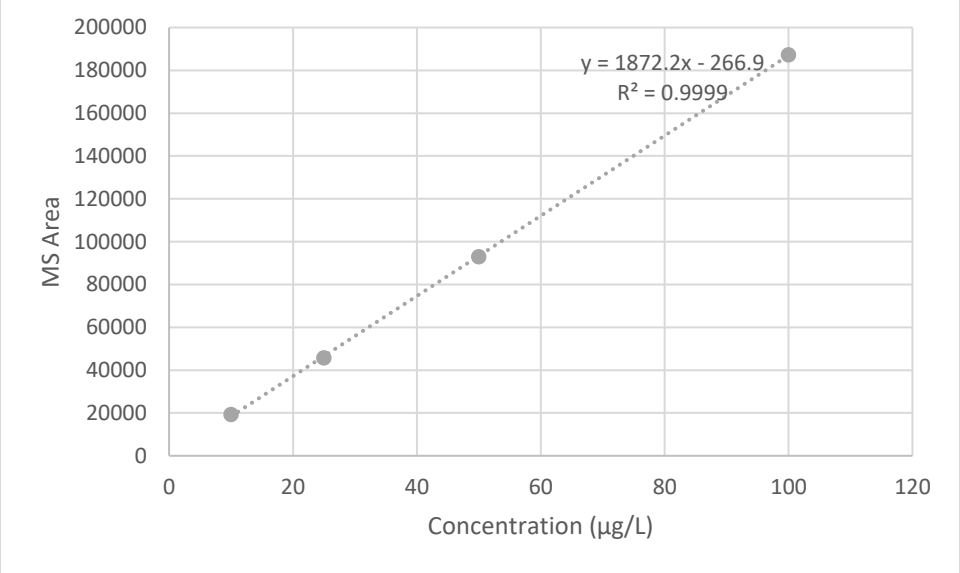


Figure A38 5,6DMBTZ calibration curve for 01/23/2020 samples.

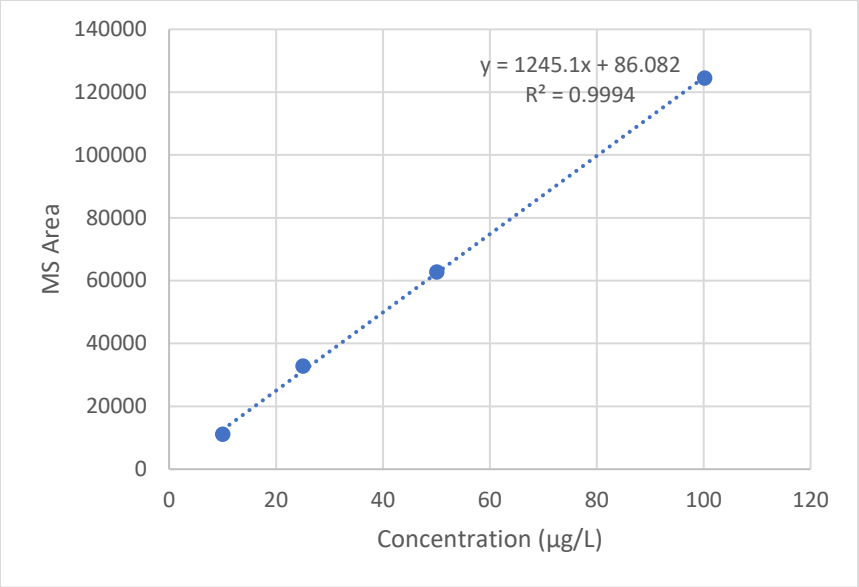


Figure A39 TTZ calibration curve for 02/11/2020 samples.

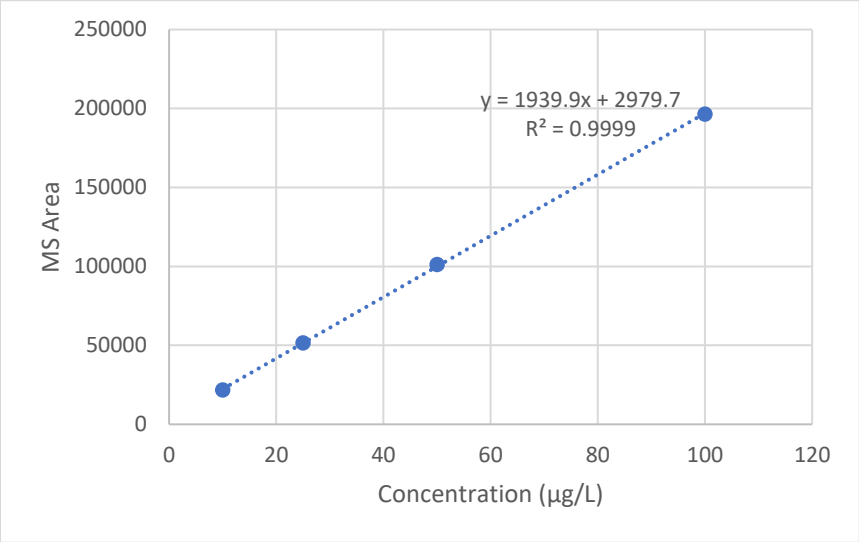


Figure A40 5,6DMBTZ calibration curve for 02/11/2020 samples.

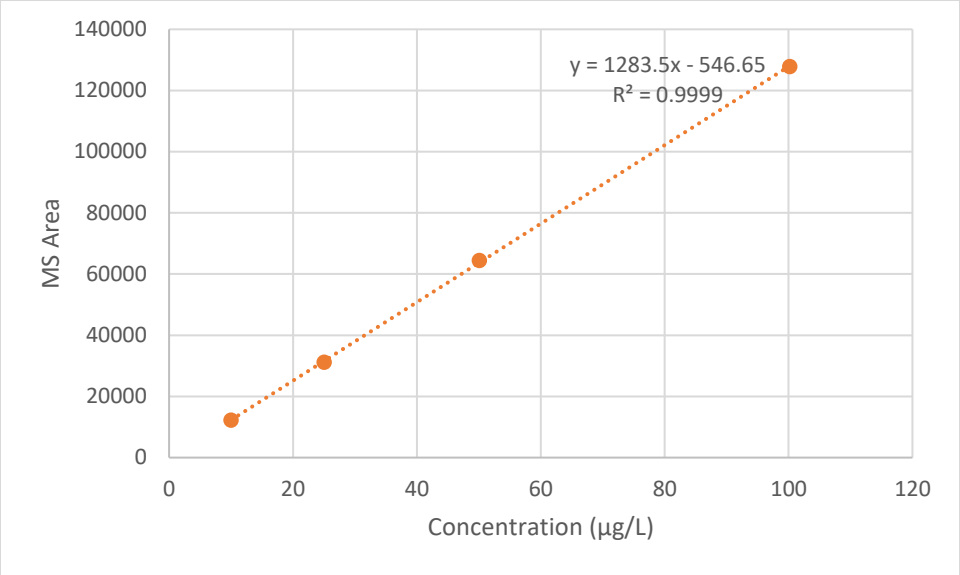


Figure A41 TTZ calibration curve for 02/25/2020 samples.

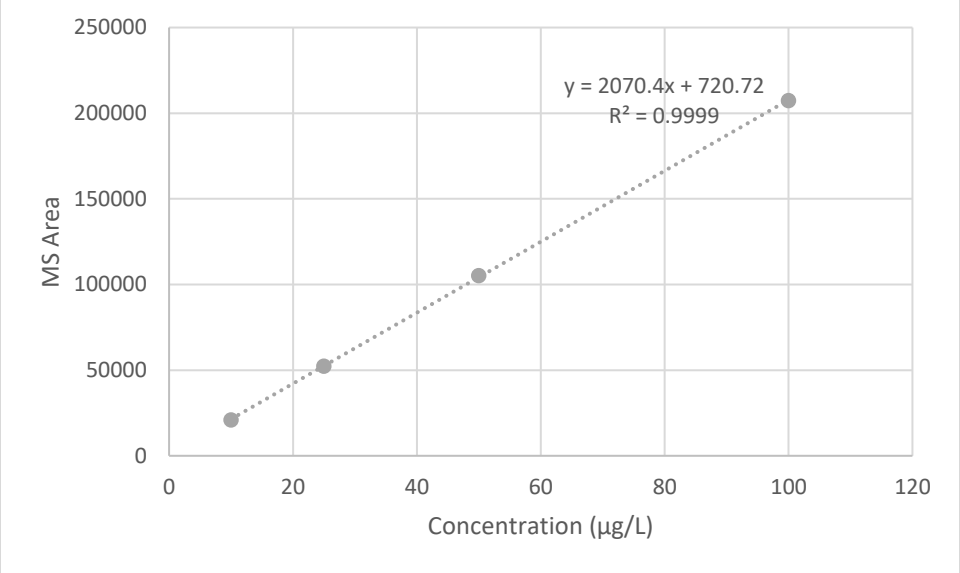


Figure A42 5,6DMBTZ calibration curve for 02/25/2020 samples.

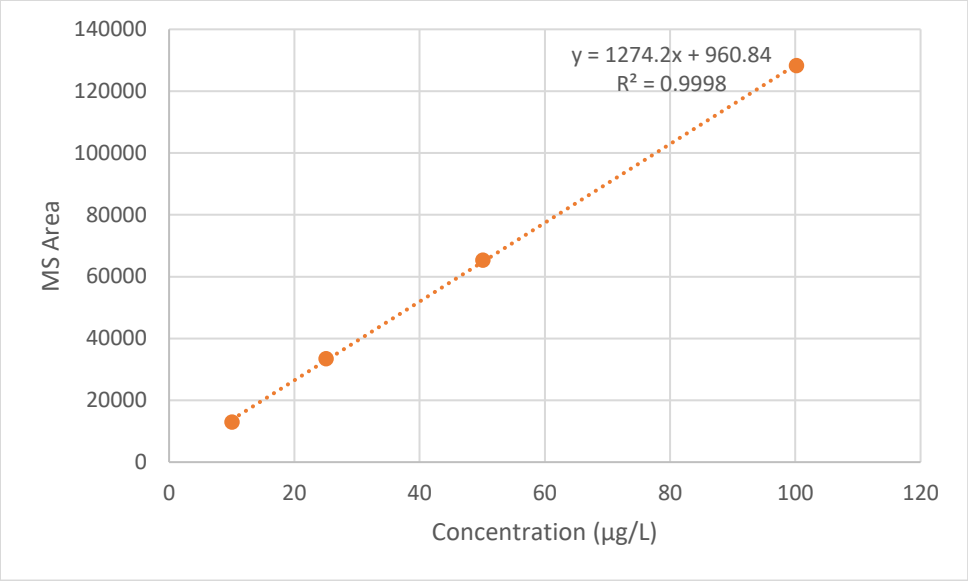


Figure A43 TTZ calibration curve for 03/10/2020 CCJKR and LCFR samples.

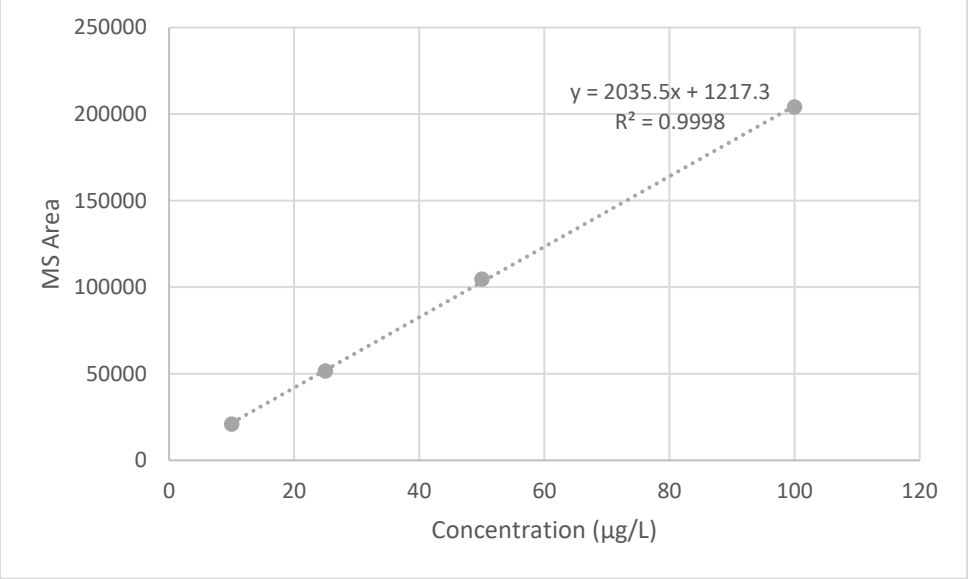


Figure A44 5,6DMBTZ calibration curve for 03/10/2020 CCJKR and LCFR samples.

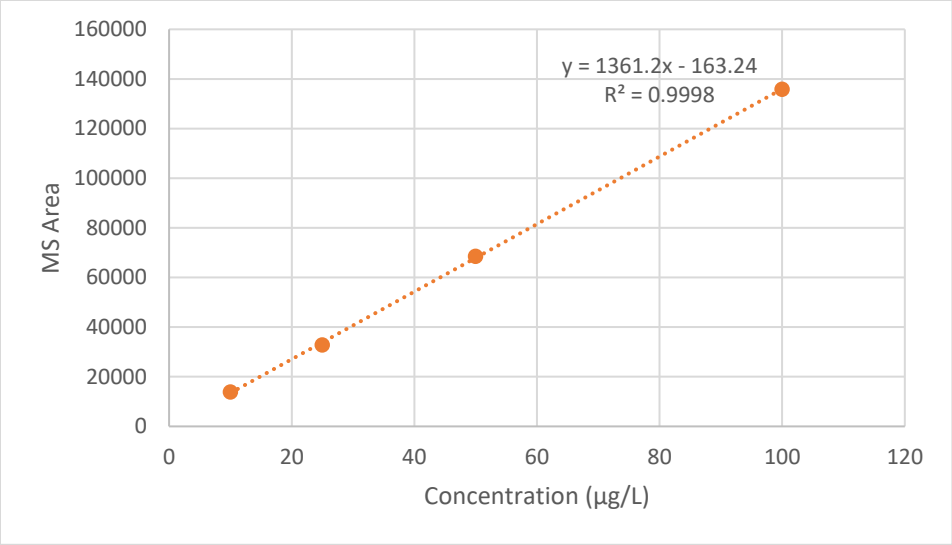


Figure A45 TTZ calibration curve for 03/10/2020 IRJKR samples.

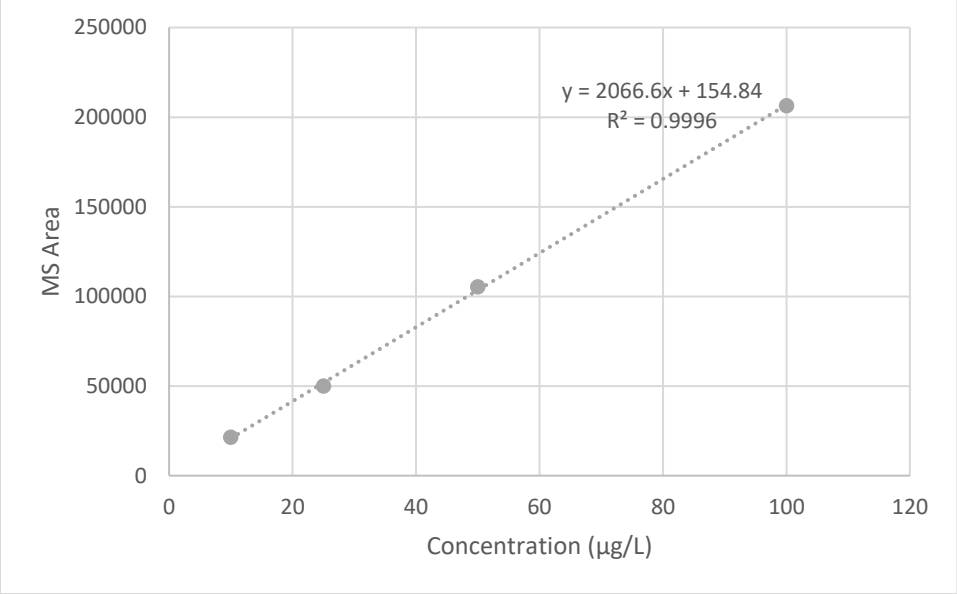


Figure A46 5,6DMBTZ calibration curve for 03/10/2020 IRJKR samples.

Appendix C

Standard Operating Procedure

ISOLATION OF BENZOTRIAZOLE AND ANALOG COMPOUNDS IN WILMINGTON AIR PARK RUNOFF WATER SAMPLES VIA SOLID-PHASE EXTRACTION

October 8, 2019

Audrey McGowin, PhD

Jessica Wiese

A. SCOPE AND APPLICATION

1H-benzotriazoles are complexing agents that are widely used as anti-corrosives, engine coolants, aircraft de-icers, anti-freezing liquids, and silver protection in dishwashing agents. Chemically, 1H-benzotriazoles are soluble in water, resistant to biodegradation, only partially removed in wastewater treatment, and have the potential to pass drinking water treatment. Most benzotriazole (BTri) compounds and their analogs are polar and thermally labile. In addition, BTris are toxic to certain aquatic organisms, and have the potential for impacting the health of creeks, rivers, and ground water reservoirs in which BTri and BTri analogs are deposited. The procedures outlined in this SOP were created for the solid-phase extraction of surface and ground water samples collected near Wilmington Air Park.

B. SUMMARY OF METHOD

The purpose of this Standard Operating Procedure (SOP) is to establish a procedure for the solid-phase extraction of surface and ground water samples collected near Wilmington Air Park in order to determine the presence of 1H-benzotriazoles, tolytriazoles, and comparable analogs in runoff from the airport's wastewater treatment plants.

C. HEALTH AND SAFETY

The analyst must assume that all surface and ground water samples are potentially contaminated and should be treated accordingly. Personal protection equipment (PPE) should be worn at all times while in the lab; this includes lab coat, protective gloves, safety glasses, long pants and closed-toe shoes.

D. SAFETY AND CAUTIONS

1. All personnel must abide by the safety procedures discussed in the "Wright State University Chemical Hygiene Plan". Any spills or emergency or accidents must be reported to the department of Environmental Health and Safety at Wright State University for assistance.

2. Material safety data sheets for all chemical reagents are available and should be read and understood by all personnel performing the methods described herein.

3. Do not pour any reagents down the drain. Collect all waste materials for proper disposal in the lab in appropriately labeled waste containers.

4. All personnel must wear a lab coat, gloves and appropriate eye protection when in the laboratory, including visitors.

5. Glassware and containers must be labeled with the chemical, the date, its concentration, hazard (if any), and the initials of the personnel responsible.

6. Final extracted sample containers must be labeled according to the Sample Labeling Scheme outlined in Section F of this SOP.

E. EQUIPMENT AND SUPPLIES

1. Laboratory notebook with ink pen

2. Permanent marker for labeling glassware/containers

3. Proper attire for lab work: lab coat, eye protection, long pants, closed-toed shoes

4. Glassware & Extraction Materials

a. Various beakers and flasks for collection/storage

b. Several glass Pasteur pipettes

c. 0.7-μm glass fiber filters (Whatman, GF/F, 47 mm)

d. Whatman 47 mm glass filter funnel and 1L Erlenmeyer flask with vacuum attachment
e. Oasis® PRIME HLB cartridges (Waters, 500 mg, 6 mL)

f. 12-port vacuum extraction manifold

g. 15-mL centrifuge tubes for eluate collection

h. Tank of nitrogen gas

i. Amber vials for storage of excess filtrates

5. Chemicals & Reagents

a. HPLC-Grade Methanol (MeOH, CAS #67-56-1)

b. Water (Milli-Q purified)

c. Hydrochloric acid (12 M HCl, CAS #7647-01-0)

d. Dichloromethane (DCM, CAS #75-09-2)

e. 5,6-dimethyl-1H-benzotriazole (5,6-dimethyl-BTri, CAS #4184-79-6)

F. SAMPLE LABELING SCHEME

Final extractions of samples will be labeled according to the following scheme:

Date (MMDDYYYY)– Sample Site – Depth – BTri – Sample Replicate Number (if needed)– Analysis Replicate Number (if needed)

For example: 10312018 – LCFR – 0 – BTri – R1-A

G. SOLID PHASE EXTRACTION PROCEDURE

1. Filter each water sample through the glass fiber filters using the funnel/flask assembly.

2. Divide each filtrate into three 100-mL replicates.

3. Acidify the replicates to pH 2.5-3.0 using 3 drops of the 12 M HCl solution.53

4. Spike each replicate with 54.0 ng (10 μL of a 5.0 ppm solution) of 5,6-dimethylBTri as the surrogate standard.

5. Connect the SPE cartridges to the ports on the vacuum extraction manifold.

6. Condition the SPE cartridges sequentially with 3 x 2 mL of MeOH and then 3 x 2 mL of Milli-Q water, applying a slight vacuum (about 5 psi).

7. Run the samples through the cartridges at a flow rate of 5 mL/min.

8. Dry the cartridges under a vacuum (15 psi) for 2 hours and 30 minutes.

9. Dissemble the vacuum extraction manifold and dispose of the water into a waste beaker; place the centrifuge tubes in the clamps beneath the ports and then reassemble the manifold. 10. Elute the analytes under a slight vacuum (5 psi) with 5 mL of DCM containing 3% MeOH, then remove the centrifuge tubes from the manifold.

11. Evaporate the eluates to dryness under a gentle stream of nitrogen gas.

12. Redissolve the dry residues in the centrifuge tubes by adding 1 mL of MeOH; store the samples in the tubes at -20 ℃ overnight.

Table A2 List of fractured or intact amber sample bottles.

Sample Day	Site-Replicate	Intact	Fractured
11132019	CCJKR-R1	X	
	CCJKR-R2	X	
	LCFR-R1	X	
	LCFR-R2		X
	IRJKR-R1	X	
	IRJKR-R2	X	
11202019	CCJKR-R1	X	
	CCJKR-R2	X	
	LCFR-R1	X	
	LCFR-R2	X	
	IRJKR-R1	X	
	IRJKR-R2		X
12042019	CCJKR-R1	X	
	LCFR-R1	X	
	IRJKR-R1	X	
01142020	CCJKR-R1	X	
	CCJKR-R2	X	
	LCFR-R1		X
	LCFR-R2		X
	IRJKR-R1	X	
	IRJKR-R2		X
01232020	CCJKR-R1	X	
	CCJKR-R2		X
	LCFR-R1	X	
	LCFR-R2		X
	IRJKR-R1	X	
	IRJKR-R2		X
02112020	CCJKR-R1	X	
	CCJKR-R2	X	
	LCFR-R1		X
	LCFR-R2	X	
	IRJKR-R1	X	
	IRJKR-R2		X
02252020	CCJKR-R1	X	
	CCJKR-R2	X	
	LCFR-R1	X	
	LCFR-R2		X
	IRJKR-R1	X	
	IRJKR-R2	X	
03102020	CCJKR-R1	X	
	CCJKR-R2	X	
	LCFR-R1	X	
	LCFR-R2	X	
	IRJKR-R1	X	
	IRJKR-R2	X	

APPENDIX D

Weather Data According to the National Weather Service.[26]

Table A3 November weather prior to and day of sampling. [26]

Date	Precipitation (in)	Temperature (F)	Weather Characteristics
11/10/2019	0	60-35, 48	Some Clouds, Fog and Haze
11/11/2019	0.18	61-26, 20	Almost completely Overcast, Fog
11/12/2019	0.02	26-14, 20	Overcast, Wind, Fog and Haze
11/13/2019	0	27-6, 17	
11/16/2019	0	41-25, 33	Some Clouds
11/17/2019	0	50-23, 37	Clear Sky, Fog and Haze
11/18/2019	0	46-29, 38	Mostly Overcast, Fog and Haze
11/19/2019	Not enough to measure	50-36, 43	Overcast, Fog

Table A4 December weather prior to and day of sampling. [26]

Date	Precipitation (in)	Temperature (F)	Weather Characteristics
12/01/2019	0.16	35-55, 45	Very overcast, Fog
12/02/2019	0.06	33-37, 35	Completely Overcast, Fog
12/03/2019	Not enough to measure	31-33, 32	Extremely overcast, Fog
12/04/2019	0	30-46, 38	Mostly clear sky,

Table A5 January weather prior to and day of sampling. [26]

Date	Precipitation (in)	Temperature (F)	Weather Characteristics
01/11/2020	0.49	66-51, 59	Overcast, Fog, Windy
01/12/2020	Not enough to measure	51-37, 44	Very Overcast, Fog, Windy
01/13/2020	0	49-36, 43	Partly Overcast
01/14/2020	0	50-30, 40	Partly Overcast, Thick Fog
01/20/2020	Not enough to measure	26-14, 20	Pretty Overcast
01/21/2020	0	28-12, 20	Little Cloud
01/22/2020	0	37-16, 27	Clear
01/23/2020	0.01	46-29, 38	Partly Overcast

Table A6 February weather prior to and day of sampling. [26]

Date	Precipitation (in)	Temperature (F)	Weather Characteristics
02/08/2020	0.06	33-26, 30	Completely Overcast, Fog
02/09/2020	0.08	42-21, 32	Very Overcast, Thick Fog, Haze
02/10/2020	0.10	47-36, 42	Completely Overcast, Fog, Windy
02/11/2020	0.04	39-32, 36	Very Overcast, Fog, Haze
02/22/2020	0	46-20, 33	Clear, Windy
02/23/2020	0	50-28, 39	Partly Cloudy
02/24/2020	0.46	43-34, 39	Pretty Overcast, Fog
02/25/2020	0.30	44-37, 41	Very Overcast, Thick Fog

Table A7 March weather prior to and day of sampling. [26]

Date	Precipitation (in)	Temperature (F)	Weather Characteristics
03/07/2020	0	47-30, 39	Some Clouds
03/08/2020	0	61-31, 46	Clear
03/09/2020	Not enough to Measure	63-44, 54	Light Cloud, Windy
03/10/2020	0.44	57-42, 50	Completely Overcast, Fog, Windy

Figure A47 First sample day, unknown field (11/13/2020)

Figure A48 Racoon prints, Lytle Creek (01/23/2020)

Figure A49 Clara Leedy and Lee Raska Cowan Creek Sample Site (03/10/2020)

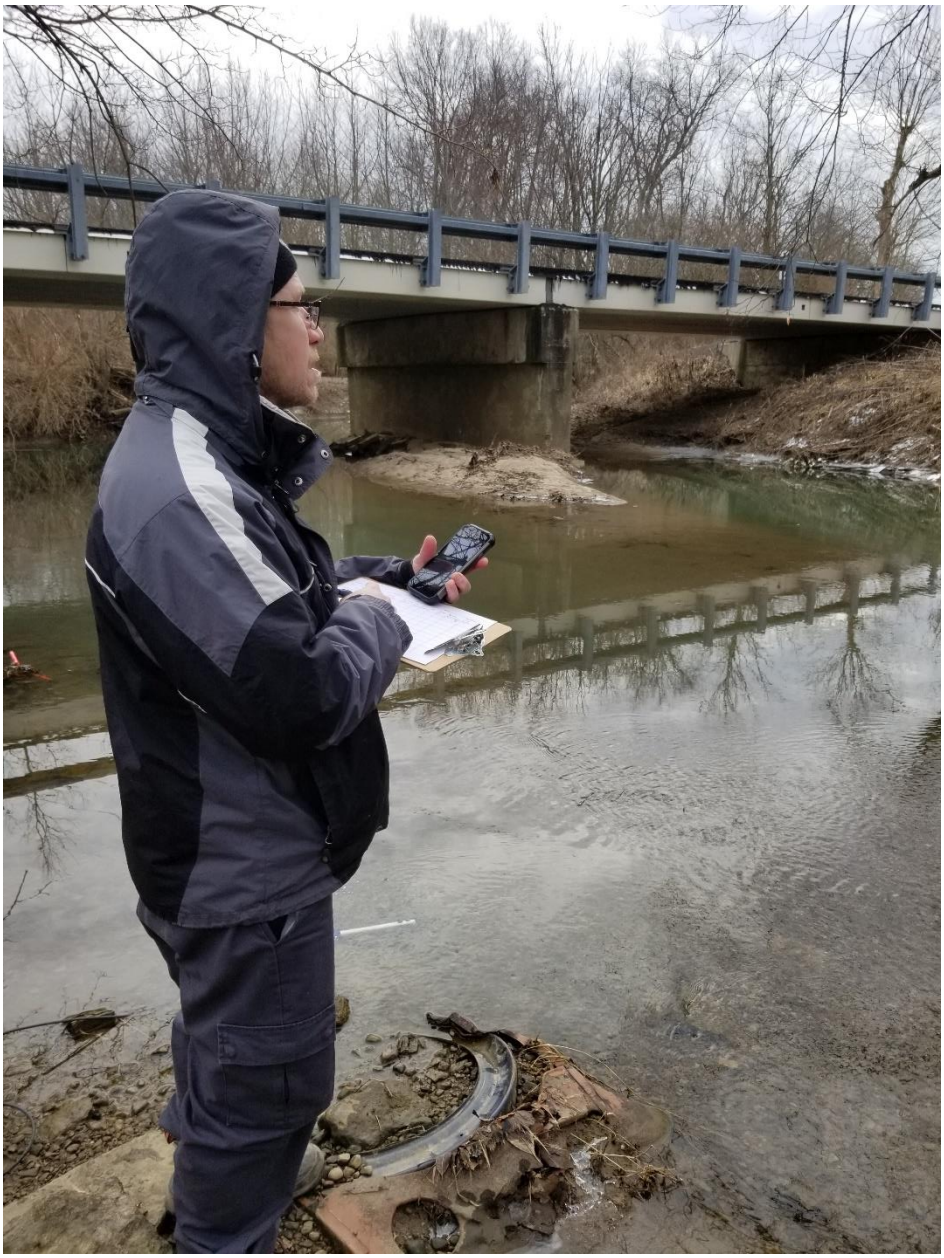


Figure A50

Travis Luncan Cowan Creek Sample Site (01/23/2020)

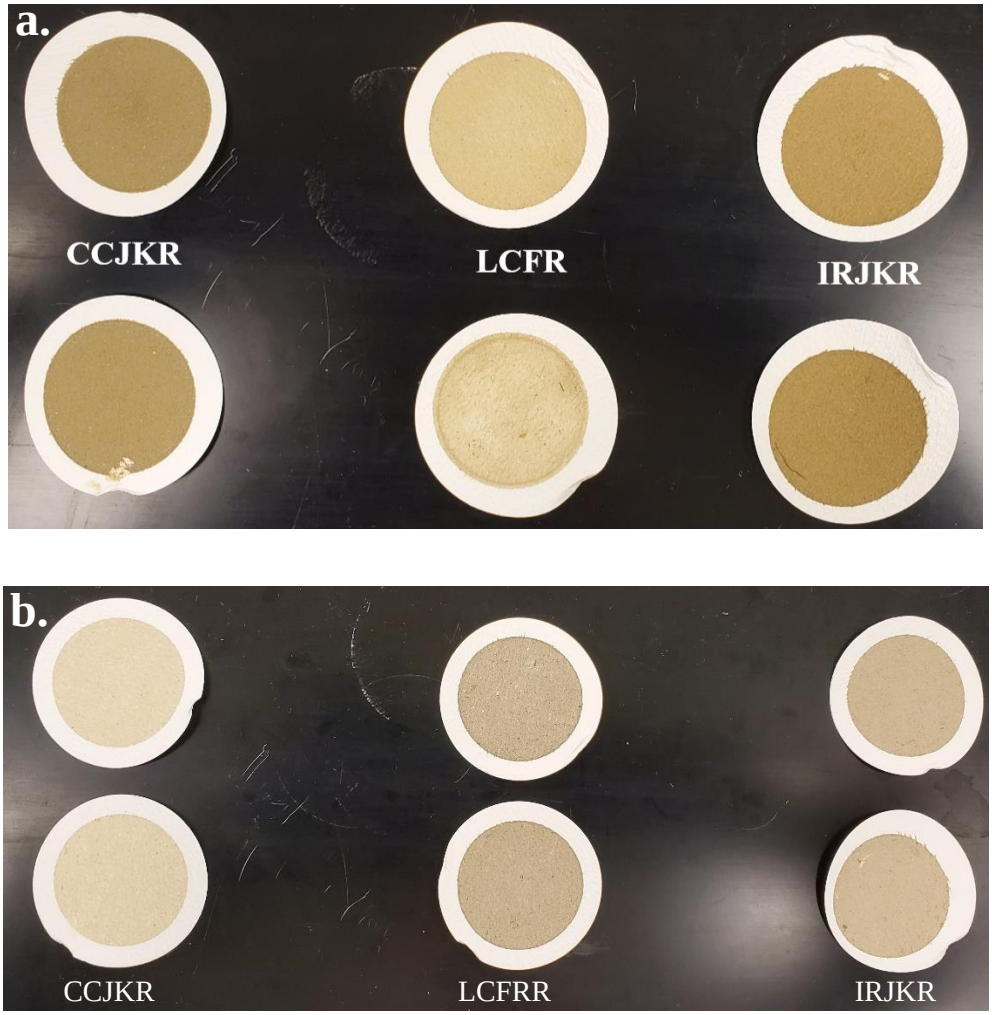


Figure A51 Filters used for sample sites procured from: a. 02-25-2020 and b. 03-10-2020

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