Native woody species response to in-water triclopyr application

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ABSTRACT

Triclopyr was first registered for use in aquatics as the water-soluble triethylamine salt formulation for the control of emersed, submersed, and floating plants. Recently, the oil- and water-soluble triclopyr acid formulation was registered for aquatic use, which allows for basal bark applications to woody plants in sites where standing water is present. Although this has greatly increased applicator flexibility to use basal bark treatments in and around water, field observations of injury to the nontarget species red maple (Acer rubrum L.) and sugarberry (Celtis laevigata Willd.) have been reported following applications to nearby Schinus tere*binthifolia* during periods of inundation. However, sensitivity of these species to triclopyr that has moved into the water following basal bark treatment is not well understood. Therefore, in-water dose-response studies were conducted in 2021 and 2022 to assess sensitivity of the nontarget species A. rubrum, C. laevigata, and buttonbush (Cephalanthus occi*dentalis* L.), to seven triclopyr concentrations ranging from 0.008 to 125 mg L^{-1} with an exposure time of 21 d. The effective dose for 50% defoliation (ED₅₀) at 49 d after treatment was 0.15, 0.385 and 1.49 mg L^{-1} for *C. laevigata*, *A.* rubrum, and C. occidentalis, respectively. Longer-term ED₅₀ values for reduction in live cambium tissue were 0.925, 1.408, and 2.519 mg L^{-1} for *A. rubrum*, *C. laevigata*, and *C. occi*dentalis, respectively. Effective doses for 15% defoliation and cambium loss were lower across species and ranged from 0.011 to 1.168 mg L^{-1} . These data indicate the potential for nontarget damage when triclopyr is present in the water. Additionally, the triclopyr acid concentration ranges tested that resulted in nontarget damage also fall within triclopyr label recommendations for in-water applications of 0.75 to 2.5 mg L^{-1} . These suggest caution for wetland and aquatic

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applicators using the triclopyr acid formulation when these desirable nontarget species are present.

Key words: aquatics, auxin herbicides, buttonbush (*Cephalanthus occidentalis* L.), herbicide injury, invasive plant management, red maple (*Acer rubrum* L.), sugarberry (*Celtis laevigata* Willd.), wetlands

INTRODUCTION

Triclopyr is a pyridine carboxylic acid herbicide used for selective weed control in both upland and aquatic sites (Shaner 2014). It is classified as a Weed Science Society of America (WSSA) Group 4 synthetic auxin and disrupts a plant's hormonal balance and natural growth processes, leading to abnormal cellular growth and leaf formation, stem twisting and swelling, and death (Shaner 2014). Three triclopyr formulations are registered for aquatic use and include the water-soluble triethylamine and choline salts, and the oil- and water-soluble acid (Anonymous 2016a, 2016b, 2017). Aquatic and wetland studies for triclopyr have generally been limited to the triethylamine salt formulation, which has demonstrated utility in aquatic and wetland sites. Foliar applications have been shown to control floating plants such as water hyacinth [Eichhornia crassipes (Mart.) Solms] and emergent plants such as purple loosestrife (Lythrum salicaria L.) (Gabor et al. 1995, Mudge and Getsinger 2019, Mudge and Netherland 2014).

In-water applications of triclopyr have also been effective for controlling certain submersed aquatic plants, but the concentration-exposure time (CET), i.e., the time required for a plant to be exposed to a given herbicide dose that results in control, varies by species. For example, Eurasian watermilfoil (*Myriophyllum spicatum* L.) was controlled with inwater applications of triclopyr amine at 2.5 mg L⁻¹ at 30 and 36 h of exposure, 2.0 mg L⁻¹ with 36 h of exposure, 1.5 mg L⁻¹ with 48 h of exposure, and 0.5 mg L⁻¹ with an exposure time of 84 h (Netherland and Getsinger 1992). In contrast, submersed applications of triclopyr amine as low as 1.25 mg L⁻¹ with an exposure time of 48 h reduced the biomass of parrotfeather [*Myriophyllum aquaticum* (Vell.) Verdc.] by 80% (Wersal and Madsen 2010).

Although CET can influence both efficacy and selectivity, triclopyr nontarget injury with different application methods has not been well quantified, especially for in-water activity. This issue has recently arisen regarding the labeled use patterns for triclopyr acid in wetlands. Due to its full aquatic label and intermediate solubility in both oil and water, basal bark applications to woody species can now be made in wetlands when standing water is present (Anonymous 2017). This

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was not previously allowable for basal bark application with triclopyr butoxyethyl ester, which can only be used in uplands and seasonally dry wetlands (Anonymous 2018). While this approach is effective for the control of invasive plants such as Brazilian peppertree (*Schinus terebinthifolia* Raddi) (Bell et al. 2023), observations of nontarget injury to red maple (*Acer rubrum* L.) and sugarberry (*Celtis laevigata* Willd.) have been reported in wetlands following basal bark applications of triclopyr acid to nearby invasive trees (Jon Morton, U.S Army Corp of Engineers, personal communication). Mesocosm studies then confirmed triclopyr acid release from basal bark treated stems and subsequent nontarget damage to *A. rubrum*, *C. laevigata*, and buttonbush (*Cephalanthus occidentalis* L.) in flooded conditions (Oberweger et al. 2023).

In general, Turner et al. (2020) found several tree species to vary in their susceptibility to foliar applications of triclopyr. Dias et al. (2017) found the activity of triclopyr formulations to differ on an interspecies level and attributed the results to potential differences in absorption and translocation. Similarly, Douglass et al. (2016) suggested more research is needed to develop triclopyr dose-response models that encompass a broader selection of plant species. This would be useful for land and aquatic managers in understanding the impacts and risks for nontarget injury during applications of triclopyr in aquatic and wetland sites.

Given these issues, our objective was to evaluate the response of *A. rubrum, C. laevigata*, and *C. occidentalis* to inwater applications of triclopyr acid though dose response studies. Our approach was designed to simulate a wetland situation where roots were exposed to triclopyr acid at various concentrations during a flooding event. Understanding the susceptibility of these common native species to triclopyr acid may improve our understanding of potential non-target risks for basal bark applications when standing water is present.

MATERIALS AND METHODS

Greenhouse dose-response experiments were conducted at the University of Florida's Center for Aquatic and Invasive Plants (29.72017°N; 82.41563°W), to evaluate the response of A. rubrum, C. laevigata, and C. occidentalis to inwater applications of triclopyr acid. In the spring of 2021, 6-mo-old, 30 to 60 cm saplings of A. rubrum, C. laevigata, and *C. occidentalis* were acquired from a local nursery.¹ The roots of each plant were thoroughly washed to remove all organic matter. Plants were transplanted into 3.8 L pots² filled with pure builder's sand³ amended with 0.5 g of slow-release fertilizer per pot.⁴ The removal of organic matter from plant roots and the selection of pure builder's sand as the substrate were intended to minimize the potential binding of triclopyr acid to organic matter. All plants were housed in a greenhouse maintained at approximately 26 C and covered with a 50% shade cloth. Plants were irrigated daily via overhead irrigation and allowed to acclimate for 1 mo.

Following acclimation, dose response studies were initiated on each species. Each experiment was set up as a completely randomized design with four replications per treatment and was conducted twice during the period of July 2021 to August 2022. Thirty-two uniform plants of each



Figure 1. Treatment approach for triclopyr dose-response trials. Plants were exposed to in-water concentrations of triclopyr via water exchange through the drainage holes around the bottom of each pot.

species were selected for each experimental run. Four pots of each species were then placed in eight (76 L) concrete mixing tubs (61 cm width by 91 cm length by 20 cm deep) (Figure 1). Treatment of the first and second experimental runs for A. rubrum took place on July 5, 2021, and August 26, 2021, respectively. Treatment of the first and second experimental runs for C. occidentalis occurred on August 26, 2021, and April 20, 2022, respectively. Treatment of the first and second experimental runs for C. laevigata took place on July 8, 2021, and September 27, 2021, respectively. Because of time and space limitations, the experimental run performed on August 26 contained four A. rubrum pots and four C. occidentalis pots per mesocosm. All other runs were performed separately for each species. Mesocosms were placed in a glasshouse at approximately 26 C for both experimental runs of A. rubrum and C. occidentalis. For C. laevigata, mesocosms were placed in a glasshouse for the first experimental run and a polyethylene plastic greenhouse for the second experimental run because of pest-related issues. Plants were maintained in tubs and subirrigated from 0 to 21 d after treatment (DAT). Plants were then removed from tubs and watered via overhead irrigation from 22 to 112 DAT.

Seven concentrations of triclopyr acid⁵ were tested on each species. These included 0.008, 0.04, 0.2, 1.0, 5.0, 25.0, and 125.0 mg L^{-1} and a nontreated control. Plants were temporarily removed from all mesocosms for treatment. Each mesocosm was filled with 32 L of well water. The herbicide was injected via micropipette into the water column of each tub to achieve a target concentration. The herbicide solution in each tub was thoroughly stirred with a glass mixing rod to ensure that it had fully diluted to a uniform concentration. Plants were then returned to their respective tubs. Pots were submersed to a depth of 13 cm, which allowed for water exchange with the tub through the drain holes at the bottom of each pot (Figure 1). Once the herbicide solution and all replicates were present, the water level of each tub was marked to designate the precise volume of water at treatment level. The water level in each tub was then maintained for 21 DAT by adding water when needed. All tubs and herbicide solutions were removed 21 DAT, and plants were returned to the benchtop in the greenhouse. Overhead irrigation of all replicates proceeded until the experiment's conclusion at 112 DAT.

Visual estimates of percent defoliation were conducted at 49 DAT. Defoliation was estimated for each plant species and was based on a scale of 0 to 100%, where 0% indicated no loss of foliage and 100% indicated a complete loss of foliage. Water sampling was conducted within 4 h after treatment and at 7 and 21 DAT to quantify triclopyr treatment concentrations. Surface water samples were collected by hand from each tub in 50 ml vials. All vials were placed on ice immediately upon collection and preserved in a freezer at -20 C until analysis for triclopyr concentration. At 112 DAT, cambium height measurements were recorded for each replicate by gently removing the outer rhytidome via a scalping tool to reveal the cambium layer. Live cambium and phloem tissue in saplings of all three species holds a distinctive light green hue and is shiny due to water content. In contrast, deceased tissue lacks this hue, appearing dry and discolored. Measurements of the total plant height and height of live cambium tissue were used to calculate the percent reduction in live cambium height.

Triclopyr acid concentrations from in-water samples were quantified using a novel liquid chromatography direct injection method. Sample preparation for this method involved filtering 5 ml of each water sample through a Millex-GV PVDF, 0.22 μ m syringe filter⁶ and transferring 1 ml of the filtered samples into 2 ml autosampler glass vials. Fifty microliters of Optima grade formic acid were added to each 2 ml glass vial concluding sample preparation. A limit of detection (LOD) of 5 μ g L⁻¹ was achieved with this direct injection method.

Samples were analyzed using a 1 µl injection volume per sample on an Agilent 1290 ultra high-performance liquid chromatography⁷ coupled to an Agilent 6495C triple quadrupole mass spectrometer (MS). The LC was equipped with a C_{18} reversed-phase LC column (2.1 by 100 mm, 1.8 µm) with a C_{18} guard column (2.1 by 5 mm, 1.8 µm) held at 25 C.⁸ Separation was achieved using a gradient mobile phase consisting of solution A (Optima Grade LC-MS water with 5 mmol ammonium formate and 0.1% Optima Grade formic acid) and solution B (Optima Grade LC-MS methanol with 5 mmol ammonium formate and 0.1% Optima Grade formic acid). The gradient changed from 5% B to 100% B in 4.5 min, with a 1-min hold at 100% B. The column was then preconditioned at 5% B for 2 min before the next run.⁹ The MS was operated in dynamic multiple reaction monitoring mode with negative electrospray ionization using m/z 255.9 as the precursor ion, m/z 197.9 as the quantifier ion, and 195.9 as the qualifier, under a collision energy of 10 V. The retention time for triclopyr was 4.486 min.

Calibration standards ranging from 5 to 1000 µg L⁻¹ were prepared by serial dilution using powdered PESTANAL[®] grade triclopyr analytical standard⁶ dissolved in Optima LC/ MS-grade water. A calibration standard series was analyzed prior to sample analysis and again after every batch of 20 samples. Passing criteria for the calibrations were a linear slope with a $R^2 \ge 0.99$. Method blank, matrix spike, and duplicate matrix spike recovery samples were analyzed with each batch of 20 experimental samples. Passing criteria were 80% to 120% recovery of the added triclopyr in spiked samples and $\le 10\%$ concentration difference between duplicate samples. Mean recoveries (%) of triclopyr were 98.0 \pm 13 with a relative standard deviation of 13%. The method quantitation limit (MQL) was 5 µg L⁻¹.

Statistical analysis

All statistical analyses were conducted using R statistical software (R Core Team 2021). All data were assessed for the assumptions of homogeneity of variance and normality and initially subjected to analysis of variance (ANOVA) via the "agricolae" package v.1.3-5 (de Mendiburu 2021) to test for main effects and interactions between experimental runs. An experimenter error resulted in the loss of the 0.2 mg L^{-1} treatment for *C. occidentalis* in the first experimental run; there were no other issues for any other treatments in either experimental run. Following this, experimental runs were pooled for dose response analysis.

Defoliation data from 49 DAT and cambium data from 112 DAT were individually regressed over triclopyr acid concentrations (mg L^{-1}) for each species and were fitted to a two-parameter log-logistic model with a lower limit of zero and an upper limit of 100 (Equation 1) using the package "drc" in RStudio and the function "drm() "to estimate the effective dose (ED) at 50% of the sample population (v. 3.0.1; Ritz et al. 2015):

$$y = c + \frac{100 - 0}{\left(1 + \exp\{b[\log(x) - \log(e)]\}\right)},$$
[1]

where y is the response (defoliation for the first analysis, cambium loss for the second analysis), x is the explanatory variable (concentration of triclopyr acid), b is the slope of the curve, and e is effective triclopyr concentration resulting in 50% defoliation or cambium loss. Although nontarget injury criteria can often be vague, for woody species in wetlands, we consider 50% defoliation or cambium reduction to be severe injury that would be unacceptable to managers. Additionally, an ED_{15} dose was calculated to assess minor, but noticeable injury similar to herbicide flashback that is approaching a threshold of concern for woody



Figure 2. Average concentrations of triclopyr acid in-water samples from treatment tubs collected at 0 d after treatment (orange), 7 d after treatment (green), and 21 d after treatment (blue). Applied concentrations of triclopyr acid (mg L^{-1}) are given on the *x* axis, and observed concentrations are represented on the *y* axis. Bars represent one standard error. The *y* axis is broken to account for extreme differences between high and low concentrations.

species (Chris Marble, personal communication). Figures were generated using the package "ggplot2" in RStudio v. 3.3.6 (Wickham 2016).

RESULTS AND DISCUSSION

Within 4 h of treatment, observed in-water triclopyr acid concentrations were lower than applied for all concentrations tested (Figure 2). This suggests rapid equilibration of triclopyr acid between the water column and sand in each pot. Triclopyr acid in-water concentrations remained relatively stable at 7 and 21 d after treatment. This is likely because of a combination of light attenuation in the greenhouse and limited microbial activity within the mesocosms, where a sand culture was used and all organic matter was washed from the roots prior to planting. Although triclopyr has been shown to be subject to photolytic degradation, Petty et al. (2003) suggested that microbial processes are a greater driver of triclopyr breakdown in aquatic systems. Nonetheless, the data are indicative that treated plants were subjected to a relatively constant triclopyr concentration over the course of the inundation period.

No significant differences between experimental runs for each species were detected and data were combined for analyses. At 49 DAT, *C. laevigata* exhibited greater defoliation than *A. rubrum* across triclopyr acid concentrations ranging from 0.01 to 0.2 mg L⁻¹ and greater defoliation than *C. occidentalis* across concentrations ranging from 0.01 to 3.0 mg L⁻¹ (Figure 3). *Cephalanthus occidentalis* also exhibited greater tolerance to triclopyr acid than *A. rubrum* when exposed to concentrations ranging from 0.07 to 4.5 mg L⁻¹. *Celtis laevigata* had an ED₁₅ of 0.011 mg L⁻¹, which suggests that it is susceptible to triclopyr acid at concentrations less than 0.2 mg L⁻¹. This is notably lower than triclopyr aquatic



Figure 3. Percent defoliation of *Acer rubrum* (red), *Celtis laevigata* (green), and *Cephalanthus occidentalis* (orange) at 49 d after in-water application of triclopyr acid and an exposure period of 21 d. Data were fitted to a two-parameter log-logistic model with an upper limit of 100 and a lower limit of 0 and regressed over concentrations of triclopyr acid (mg L^{-1}). The effective dose (ED₅₀) of triclopyr acid (mg L^{-1}) to cause 50% defoliation of each species is denoted by the corresponding black symbols. Shaded regions are the 95% confidence intervals.

use patterns (Table 1). Acer rubrum required exposure to 0.095 mg L⁻¹ for 15% defoliation, suggesting greater tolerance compared to *C. laevigata* at low concentrations (Table 1). Acer rubrum and *C. laevigata* exhibited similar activity at their ED₅₀ concentrations and nearly identical responses (>75% defoliation) at and above 1.0 mg L⁻¹ (Figure 3). However, *C. occidentalis* exhibited a greater tolerance to triclopyr acid than *A. rubrum* and *C. laevigata*, as reflected by an ED₅₀ of 1.493 mg L⁻¹, the highest of all species, and considerably lower defoliation compared to *C. laevigata* and *A. rubrum* until exposure at approximately 3.9 and 4.2 mg L⁻¹, respectively (Figure 3). These results indicate that *C. occidentalis* exhibits lower defoliation (i.e., is more tolerant to triclopyr acid) than *C. laevigata* and *A. rubrum*, while *A. rubrum* appears more tolerant in the short-term than *C. laevigata* at low concentrations.

Although defoliation is a primary indicator herbicide damage, live cambium tissue loss at 112 DAT provided additional evidence of herbicidal activity in the vascular tissues of each species. The loss of live cambium tissue in C. laevigata and A. rubrum at 112 DAT did not differ (Figure 4). The estimated effective dose to generate a 50% loss of live cambium tissue (ED₅₀) ranged from 0.925 mg L⁻¹ in A. rubrum to 1.408 mg L⁻¹ in *C. laevigata*. However, the ED₁₅ and ED₅₀ estimates for cambium loss in C. laevigata were all greater than A. rubrum (Table 2). This may suggest that while low concentrations of triclopyr acid in C. laevigata result in greater initial defoliation, it may have a greater capacity to recover from injury based on its ability to retain live cambium tissue. Conversely, low concentrations result in less initial defoliation in A. rubrum compared to C. laevigata, but the effects may be more lasting due to greater eventual cambium loss. Live cambium tissue loss in C. occidentalis confirmed that this species is more tolerant to triclopyr acid. Cephalanthus occidentalis responded differently at concentrations ranging from 0.59 to 1.89 mg L^{-1} , with an ED₁₅ of 1.168 and an ED_{50} of 2.519 mg L⁻¹, the highest of all tested species (Table 2; Figure 4).

Table 1. Model parameters for percent defoliation at 49 d after treatment across both experimental runs. A two-parameter log-logistic model 1 with a lower limit of zero and an upper limit of 100 were used in the analysis. Effective dose (ED_{15} and ED_{50}) values are the estimated concentrations of triclopyr acid (mg L $^{-1}$) that result in 15% and 50% defoliation, respectively, of each species. Standard error values represented in parentheses.

Species	Slope (b)	ED_{50}	ED_{15}
Acer rubrum	-1.24 (0.24)	$0.385 (0.068) \\ 0.150 (0.050) \\ 1.493 (0.105)$	0.095 (0.032)
Celtis laevigata	-0.66 (0.13)		0.011 (0.007)
Cephalanthus occidentalis	-1.55 (0.16)		0.486 (0.064)

Table 2. Model parameters for percent cambium loss at 112 d after treatment across both experimental runs. A two-parameter log-logistic model 1 with a lower limit of zero and an upper limit of 100 were used in the analysis. Effective dose $(ED_{15} \mbox{ and } ED_{50})$ values are the estimated concentrations of triclopyr acid (mg L^{-1}) that result in 15% and 50% loss of cambium tissue, respectively, of each species. Standard error values represented in parentheses.

Species	Slope (b)	ED_{50}	ED_{15}
Acer rubrum	-1.39 (0.39) -1.46 (0.38) -2.26 (0.36)	0.925 (0.198)	0.265 (0.125)
Celtis laevigata		1.408 (0.249)	0.430 (0.156)
Cephalanthus occidentalis		2.519 (0.315)	1.168 (0.228)

¹Two-parameter log-logistic model used in the analyses: $y = c + \frac{100-0}{(1+\exp\{\delta[\log(x) - \log(c)]\})}$.

The results presented herein provide insight into the response of three native woody species exposed to in-water applications of triclopyr acid and indicate that C. occidentalis is the most tolerant of the three species tested. These results also support Hutchinson and Langeland (2010), who found that the triethylamine salt formulation of triclopyr was ineffective for C. occidentalis control. We also found that low concentrations of triclopyr acid resulted in greater defoliation of C. laevigata compared to A. rubrum, but that the cambium was more severely impacted in latter. In previous foliar studies, C. laevigata was highly susceptible to triclopyr amine (Turner et al. 2020). However, A. rubrum has exhibited intermediate sensitivity to foliar applications of triclopyr, which vary among formulations and exposure routes (Fears 1980, Forster et al. 1997, Self 2020, Turner et al. 2020).

The importance of understanding the sensitivity of these native woody species is magnified in wetlands, where intermittent flooding may result in the creation of isolated areas with little to no water exchange (Haag and Lee 2010). This may enhance conditions for triclopyr retention, whereas water exchange in lakes and flowing waters typically facilitate more rapid diffusion, dilution, and breakdown (Petty et al. 2003). Recent mesocosm work has demonstrated this



Figure 4. Percent cambium loss for *Acer rubrum* (red), *Celtis laevigata* (green), and *Cephalanthus occidentalis* (orange) at 49 d after in-water application of triclopyr acid and an exposure period of 21 d. Data were fitted to a two-parameter log-logistic model with an upper limit of 100 and a lower limit of 0 and regressed over concentrations of triclopyr acid (mg L⁻¹). The effective dose (ED₅₀) of triclopyr acid (mg L⁻¹) to cause a 50% loss of cambium tissue for each species is denoted by the corresponding black symbols. Shaded regions are the 95% confidence intervals.

¹Two-parameter log-logistic model: $y = c + \frac{100-0}{(1 + \exp\{b[\log(x) - \log(c)]\})}$.

very clearly. Oberweger et al. (2023) found triclopyr acid concentrations greater than 2 mg L^{-1} in surface waters at 21 d after triclopyr acid basal bark treatments were applied to *S. terebinthifolia* and immediately flooded. This resulted in significant nontarget damage to all three species evaluated in the current study.

In addition to the potential for triclopyr acid release from basal bark treatment in wetlands at concentrations resulting in nontarget damage, our current data indicate that all three species were susceptible to triclopyr acid concentrations that coincide with label recommendations of 0.75 to 2.5 mg L⁻¹ for in-water applications for submersed aquatic vegetation control (Anonymous 2017). As such, we recommend that wetland and aquatic managers proceed with caution when performing triclopyr applications in the vicinity of C. laevigata and A. rubrum due to their susceptibility to triclopyr acid and stress the importance of following stewardship guidelines. We agree with Douglass et al. (2016) that further research is needed to establish triclopyr doseresponse models encompassing a wider selection of plant species. This information is necessary to help land managers determine environmental impacts and balance the effectiveness of target plant control with the risk for nontarget injury. We also recommend that future research address modified basal bark application approaches to reduce triclopyr acid inputs into standing water when it is used in wetlands.

SOURCES OF MATERIALS

¹Urban Forestry Services, 301 West Seminary, Micanopy, FL 32667.

²Nursery Supply, Inc, 250 Canal Rd, Fairless Hills, PA 19030.

³Vulcan Materials Company Keuka Sand Mine, 1451 State Rte 100, Melrose, FL 32666.

⁴Osmocote[©] Smart-Release Plant Food (N-P-K = 14-14-14)], Scotts-Sierra Horticultural Products, 14111 Scottslawn Road Marysville, OH 43041.

⁵Trycera[®], Helena Agri-Enterprises, 225 Schilling Blvd, Suite 300, Collierville, TN 38017.

⁶Millipore Sigma, 400 Summit Dr, Burlington, MA 01803.

⁷Agilent Technologies, 2850 Centerville Rd, Wilmington, DE 19808.

⁸ZORBAX Eclipse Plus C₁₈, Agilent Technologies, 2850 Centerville Rd, Wilmington, DE 19808.

⁹Thermo Fisher Scientific, 168 3rd Ave, Waltham, MA 02451.

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