Application Note

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A Fast Analysis of the GC/MS/MS Amenable Pesticides Regulated by the California Bureau of Cannabis Control

Authors

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Introduction

The identification and quantification of residual pesticides in cannabis flower is required by most U.S. States and Canada where medicinal or adult recreational use of cannabis has been legalized. Although the assay and the required hardware share a singular commonality across these regions, the analytical challenges of sensitivity, specificity, accuracy, precision, and robustness are thwarted by a complex matrix, a myriad of target lists, and region-specific action levels/limits of quantitation (LOQ). Adding to this complexity is the need for sample preparation procedures that remove co-extractive interferences such as Δ^9-tetrahydrocannabinol (THC) and other cannabinoids, terpenes, polyphenols, chlorophyll, and many other chemical entities synthesized by *Cannabis spp*.

In 2018, Agilent, in conjunction with Pacific Agricultural Laboratories, published *A Novel Comprehensive Strategy for Residual Pesticide Analysis in Cannabis Flower.*[1] This was later followed by *A Sensitive and Robust Workflow to Measure Residual Pesticides and Mycotoxins from the Canadian Target List in Dry Cannabis Flower*[2] developed in Agilent's Montreal Center of Excellence using dry cannabis extracts provided by Canopy Growth Corporation—a marijuana grower based in Smiths Falls, Ontario. In March of 2019, Agilent published *Determination of Pesticides and Mycotoxins as Defined by California State Recreational Cannabis Regulations.*[3] These application notes illustrate a single-stream sample preparation procedure, and both liquid chromatography-triple quadrupole mass spectrometry (LC/MS/MS) and gas phase triple quadrupole-mass spectrometry (GC/MS/MS) for the analysis of regulated pesticides in dry cannabis matrices.

This study presents a fast-chromatographic method for the analysis of the GC/MS/MS amenable pesticides regulated by the California Bureau of Cannabis Control in dry flower extracts. These include pentachloronitrobenzene (PCNB), methyl parathion, captan, and *cis/trans*-chlordane. This methodology is to be used in conjunction with *Determination of Pesticides and Mycotoxins as Defined by California State Recreational Cannabis Regulations*[3] including the single stream sample preparation procedure with a modification of the final diluent and dilution factor specific to the GC/MS/MS analyses.

Materials and methods

An Agilent 7890B Gas Chromatograph combined with an Agilent 7010B Mass Spectrometer was used. The GC was equipped with the Multi-Mode Inlet (MMI), the Purged Ultimate Union configured for midcolumn backflush, and two 15-meter HP-5MS UI columns (p/n 19091S-431). The inlet was configured for cold, pulsed splitless injections with the Ultra-Inert 2 mm dimpled splitless liner (p/n 5190-4006), and Ultra-Inert Non-Stick Advanced Green 11 mm septa (p/n 5190-3158).

7890B GC method parameters

7693 Auto-sampler configuration: Front Injector with a 5 µL syringe. Sample wash volume 2 µL followed by three sample pumps. The injection type was two layer Sandwich (L1, L2) with L1 Airgap, L2 Volume, and L2 Airgap all set for 0.2 µL. Ten-percent concentration analyte protectants were used with the sandwiched injection. More information on the analyte protectants can be found in the appendix.

A 1.0 µL sample injection was made with a two second viscosity delay. The sample injection dispense speed was 3,000 µL/minute with no preinjection or postinjection dwell time. Four postinjection washes of wash solvent A and wash solvent B (both 100% high purity, pesticide-grade acetonitrile) at 4 µL each. The solvent wash draw and dispense speeds were 300 µL/minute, and the solvent wash mode was A1-A6, B1-B4.

Parameter	Value
MMI Program	Pulsed, splitless mode with a nominal head pressure of 19.768 psi
Septum Purge Flow	3 mL/minute in switched mode with a total flow of 54.3 mL/minute
Injection Pulse Pressure	35 psi until 0.95 minutes
Purge Flow To Split Vent	50 mL/minute at 0.98 minutes
Gas Saver	20 mL/minutes after two minutes
Cryo Gas	Air
Initial Inlet Temperature	70 °C with 0.35 minute hold time
Inlet Temperature	Ramped at 350 °C/min to the injection temperature of 280 °C
Inlet Post Run Temperature	310 °C
Post Run Total Flow	25 mL/minutes
Oven Program	70 °C for one minute, 40 °C/minute to 170 °C (0 minute hold time), 10 °C/min to 250 °C (0 minute hold time)
Post Run Temperature	280 °C
Post Run (Backflush) Time	1.24 minutes (5 void columns)
Total Analysis Time	11.5 minutes.
Total Run Time	12.74 minutes
Total Cycle Time	~15 minutes
Column 1	15 m × 250 µm, 0.25 µm HP-5MS Ultra Inert (19091S-431UI) Helium carrier gas at 1.3 mL/minute
Post Run (Backflush) Flow	–4.0296 mL/minute.
Column 2	15 m × 250 µm, 0.25 µm HP-5MS Ultra Inert (19091S-431UI) Helium carrier gas in ramped flow mode.
Column 2 Flow Program	Initially 3 mL/minute for a hold time of 0.95 minutes, reduced to 1.5 mL/minute with a 100 mL/minute ramp.
Post Run (Backflush) Flow	4.4437 mL/minute
Collision Cell	Helium quench gas 2.25 mL/minute Nitrogen collision gas 1.5 mL/minute

7010B Mass spectrometer method parameters

The solvent delay was seven minutes. The mass spectrometer was operated in electron ionization (EI) mode with a source temperature of 300 °C and a transfer line temperature of 280 °C. Table 1 presents the MRM transitions, quadrupole resolution, collision energy, and gain factor.

Table 1. MS/MS conditions on the 7010B mas spectrometer.

Time Segment	Start Time	Compound Name	ISTD?	Precursor Ion	Q1 Resolution	Product Ion	Q2 Resolution	Dwell	Collision Energy	Gain Factor
1	7	Pentachloronitrobenzene	FALSE	248.7	Wide	144	Wide	50	45	15
1	7	Pentachloronitrobenzene	FALSE	248.7	Wide	141.9	Wide	50	45	15
1	7	Pentachloronitrobenzene	FALSE	248.7	Wide	213.9	Wide	50	15	15
1	7	Pentachloronitrobenzene	FALSE	236.8	Wide	143	Wide	50	30	15
1	7	Pentachloronitrobenzene	FALSE	213.7	Wide	178.9	Wide	50	15	15
1	7	Pentachloronitrobenzene	FALSE	106.9	Wide	47	Wide	50	45	15
2	8.14	Parathion-methyl	FALSE	262.9	Wide	79	Wide	75	30	15
2	8.14	Parathion-methyl	FALSE	262.9	Wide	109	Wide	75	10	15
2	8.14	Parathion-methyl	FALSE	125	Wide	79	Wide	75	30	15
2	8.14	Parathion-methyl	FALSE	125	Wide	47	Wide	75	10	15
3	9.35	Captan-d_6	TRUE	270	Wide	84	Wide	45	25	15
3	9.35	Captan-d_6	TRUE	154	Wide	84	Wide	45	20	15
3	9.35	Captan	FALSE	151	Wide	79	Wide	45	30	15
3	9.35	Captan	FALSE	151	Wide	80	Wide	45	30	15
3	9.35	Captan	FALSE	149	Wide	79.1	Wide	45	30	15
3	9.35	Captan	FALSE	149	Wide	70	Wide	45	15	15
3	9.35	Captan	FALSE	116.9	Wide	82	Wide	45	30	15
4	10.2	Chlordane	FALSE	377	Wide	267.8	Wide	75	25	15
4	10.2	Chlordane	FALSE	375	Wide	265.8	Wide	75	25	15
4	10.2	Chlordane	FALSE	271.8	Wide	118.9	Wide	75	15	15
4	10.2	Chlordane	FALSE	196	Wide	126.1	Wide	75	25	15

Detailed sample preparation common to both LC/MS/MS and GC/MS/MS

To analyze a representative sample, the cannabis must be fully homogenized prior to its extraction. This can be done by adding two ceramic homogenizers (p/n 5982-9313) or stainless-steel beads to a tube of chopped cannabis and mechanically shaking for at least five minutes at high speed (ideally on a vertical shaking device, such as a Geno/Grinder-type machine). The homogenizers will help turn the dry cannabis into fine powder.

1. Weigh 1.0 g of homogenized cannabis sample into a 50 mL polypropylene (PP) centrifuge tube.
2. Add two ceramic homogenizers to the tube, and cap.
4. If precleanup spiked matrix samples are to be prepared, pipette the pesticide standard solution(s), isotopically labeled captan-d_6, and mycotoxin standards into the dry cannabis powder, then vortex for 30 seconds.
5. Add 15 mL of pesticide-grade acetonitrile to the tube from step 3.
6. Shake the tube mechanically for three to five minutes at high speed, ideally on a vertical shaking device (Geno/Grinder-type machine). This will extract the pesticides and mycotoxins into the acetonitrile.
7. While the tube is shaking, prepare the solid phase extraction (SPE) manifold by placing a SampliQ C18 EC 6 mL 500 mg SPE cartridge (p/n 5982-1365) onto the manifold. Place a collection tube that can hold 25 mL or more. Ideally, use a graduated 50 mL PP centrifuge tube underneath the cartridge in which the eluent will be collected.
8. Decant the supernatant from step 6 into the SampliQ C18 EC SPE cartridge. It will flow by gravity.
9. After the entire solvent has gone through the C18 cartridge and is collected, add 5 mL of acetonitrile to the empty tube from step 6, and shake mechanically for three to five minutes at high speed. This will extract pesticides and mycotoxins that may still be in the cannabis material.
10. Decant the supernatant from step 9 into the same SampliQ C18 EC SPE cartridge.
11. Rinse the empty tube from step 9 with a final 5 mL of acetonitrile to wash any pesticides that might be retained on the tube wall, then pass this solvent through the same C18 cartridge. A volume of less than 25 mL (three portions of 15, 5, and 5 mL) of acetonitrile extract is collected.
12. Transfer all eluent into a volumetric flask, bring the final volume to 25 mL with acetonitrile or use the 25 mL mark on the graduated 50 mL PP centrifuge tube to adjust to 25 mL total. Vortex. Now the sample has been diluted by a factor of 25.
13. Transfer the cleaned extract (step 12) into a clean tube, cap, and label.

Detailed sample preparation unique to GC/MS/MS

The typical Agilent sample preparation workflow for GC/MS/MS is as follows:

- Into a 2 mL vial, mix 25 fold diluted extract with 100% high purity, pesticide grade acetonitrile in a 1-to-5 proportion resulting in a 125 fold dilution factor. Vortex for 10 seconds. The sample is now ready for injection on the GC/MS/MS system.

In this study however, we endeavored to test the robustness of the inlet conditions as a function of higher matrix injections. To this end, a six-point calibration curve was constructed over the range of 8 to 250 ppb at 25-fold dilutions as given in steps 1 to 13 above. This represents 200 ppb (0.2 µg/g) to 6,250 ppb (6.25 µg/g) in the dry flower matrix.

Results and discussion

The analyte retention order was PCNB (7.587 minutes), methyl parathion (8.486 minutes), captan-d_6 (10.00 minutes), captan (10.040 minutes), cis-chlordane (10.334 minutes), and *trans*-chlordane (10.593 minutes). Figure 1 illustrates the fast GC/MS/MS MRM chromatograms for the five compounds at 8 ppb in-vial (200 ppb in dry flower matrix) and the linear calibration curves over the range of 8 through 250 ppb in the autosampler vials.

Each calibrator was injected three times, and all 18 calibrator injections were used to construct the calibration curves. Table 2 is the results summary for LOQ, LOD, %RSD, and R^2.

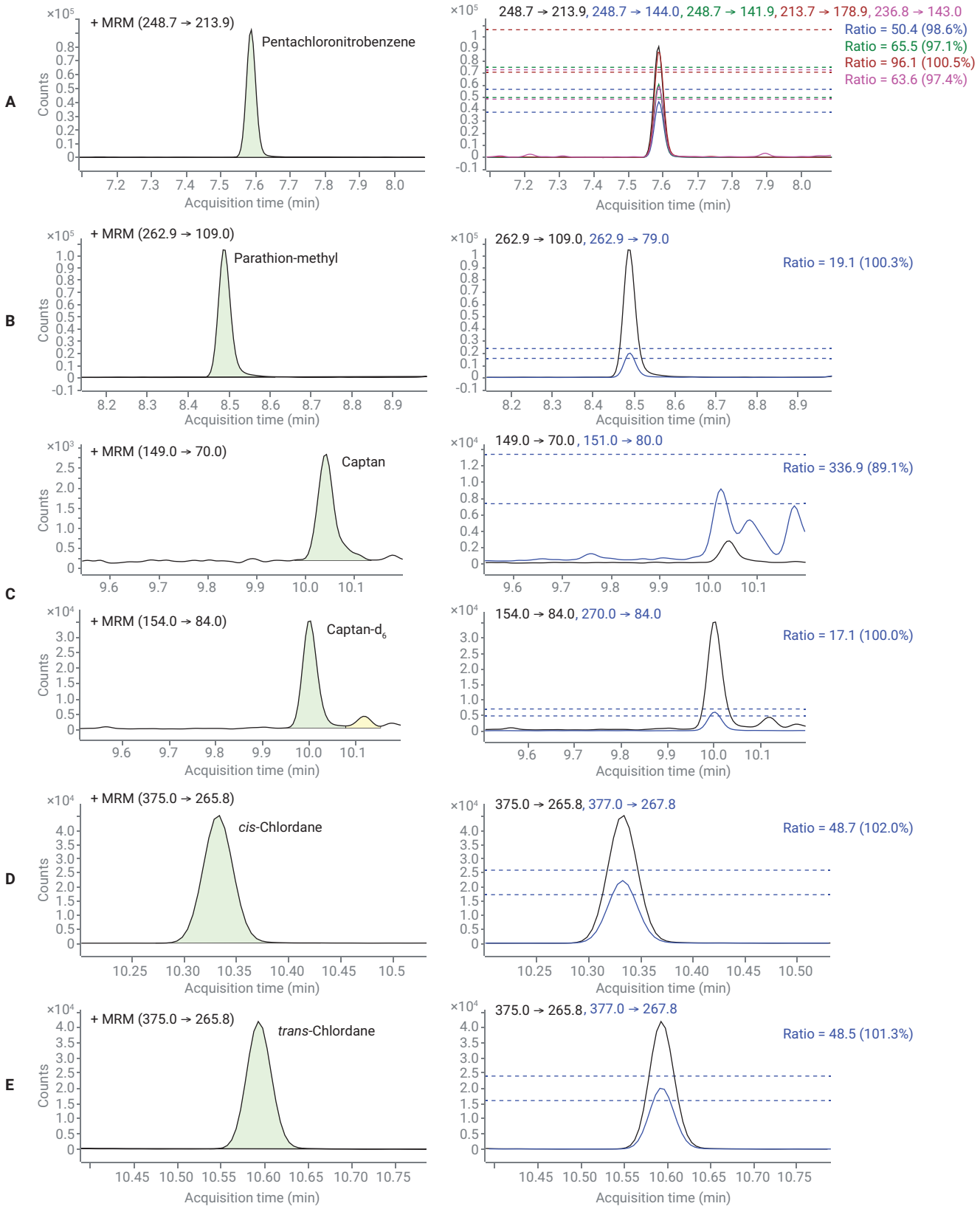

Figure 1. A: PCNB; B: methyl parathion; C: Captan and captan-d_6 internal standard: D: *cis*-chlordane; E: *trans*-chlordane.

Table 2. Results summary table. Empirical LOQ in-vial determined by a signal-to-noise ratio (S/N) of 10:1. Empirical LOD in-vial determined by a S/N of 3:1. In-matrix values determined by multiplying the in-vial values by the 25-fold dilution factor.

Compound	CA Category	CA LOQ (ppb)	Empirical LOQ In-Vial (ppb)	Empirical LOQ In-Matrix (ppb)	Empirical LOD In-Vial (ppb)	Empirical LOD In-Matrix (ppb)	%RSD	R^2
PCNB	II	100.0	0.2	4.0	0.1	1.3	0.47	>0.999
Methyl Parathion	I	>LOD	0.5	11.8	0.2	3.9	2.85	>0.994
Captan	II	700.0	4.9	122.0	1.6	40.7	0.16	>0.998
cis-Chlordane	I	>LOD	0.05	1.3	0.02	0.4	0.16	>0.999
trans-Chlordane	I	>LOD	0.06	1.6	0.02	0.5	0.78	>0.999

Conclusion

The fast GC/MS/MS method described in this Application Note illustrates the sensitivity, accuracy, and precision for the robust analysis of dry flower cannabis matrices. Challenging the inlet with the higher 25-fold matrix dilutions resulted in liner changes every 50 to 60 injections. The 200 to 6,250 ppb calibration range encompasses the action levels for captan at 700 ppb for inhalable products and 5,000 ppb for all other products.

The method should be used in conjunction with Agilent Application Note 5994-0648 specific to California including the single-stream sample preparation designed for use on both LC/MS/MS and GC/MS/MS platforms.

Acknowledgements

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References

1. Asanuma, L.; *et al.* A Novel Comprehensive Strategy for Residual Pesticide Analysis in Cannabis Flower. *Agilent Technologies Application Note*, publication number 5991-9030, **2018**.
2. Roy, J-F.; *et al.* A Sensitive and Robust Workflow to Measure Residual Pesticides and Mycotoxins from the Canadian Target List in Dry Cannabis Flower. *Agilent Technologies Application Note*, publication number 5994-0429, **2018**.
3. Stone, P. J. W.; *et al.* Determination of Pesticides and Mycotoxins as Defined by California State Recreational Cannabis Regulations. *Agilent Technologies Application Note*, publication number 5994-0648, **2019**.

Appendix

Analyte protectant preparation

- L-Gulonic acid γ-lactone (L-gulonolactone), CAS no.1128-23-0: >95% purity; Sigma-Aldrich.
- (g) D-Sorbitol, CAS no. 50-70-4: >95% purity; Sigma-Aldrich.

L-Gulonolactone stock solution

Weigh approximately 500 mg of L-gulonolactone in a 10 mL volumetric flask. Add 4 mL of water, and then bring to volume with acetonitrile. Sonicate to dissolve if needed.

(k) D-Sorbitol stock solution

Weigh approximately 500 mg of D-sorbitol in a 10 mL volumetric flask. Add 5 mL of water, and then bring to volume with acetonitrile. Sonicate to dissolve if needed.

(l) Analyte protectant (AP) solution (20 mg/mL L-gulonolactone and 10 mg/mL D-sorbitol composite solution)

Add 4 mL of the L-gulonolactone stock solution and 2 mL of the D-sorbitol stock solution into a 10 mL volumetric flask, and bring to volume with acetonitrile.

For use with the GC/MS/MS system, it is recommended to dilute this mix 1:10 in acetonitrile put in position 2 on the autosampler rotating tray. Use the standard sandwich technique. 0.1 µL air plug above and below and 0.1 µL of the protectants.

Keep refrigerated until use. Stored in the refrigerator, it lasts a month. On the rotator tray, they will break down over time and fresh solution will need to be replaced each three days on the tray or a loss of sensitivity or tailing will occur.

Setting up a sandwich injection

Place the vial containing analyte protectants in position L2 in the autosampler (2 mL in the vial) at the above concentration. Make a 2 µL injection of sample, and a 0.2 µL injection of analyte protectants. This will act as a type of matrix-matched standard. Use this to inject your standards and samples as well.

Alternatively, one can spike each vial with the analyte protectants; however, this increases sample prep (it is recommended to let the autosampler do the work for you).

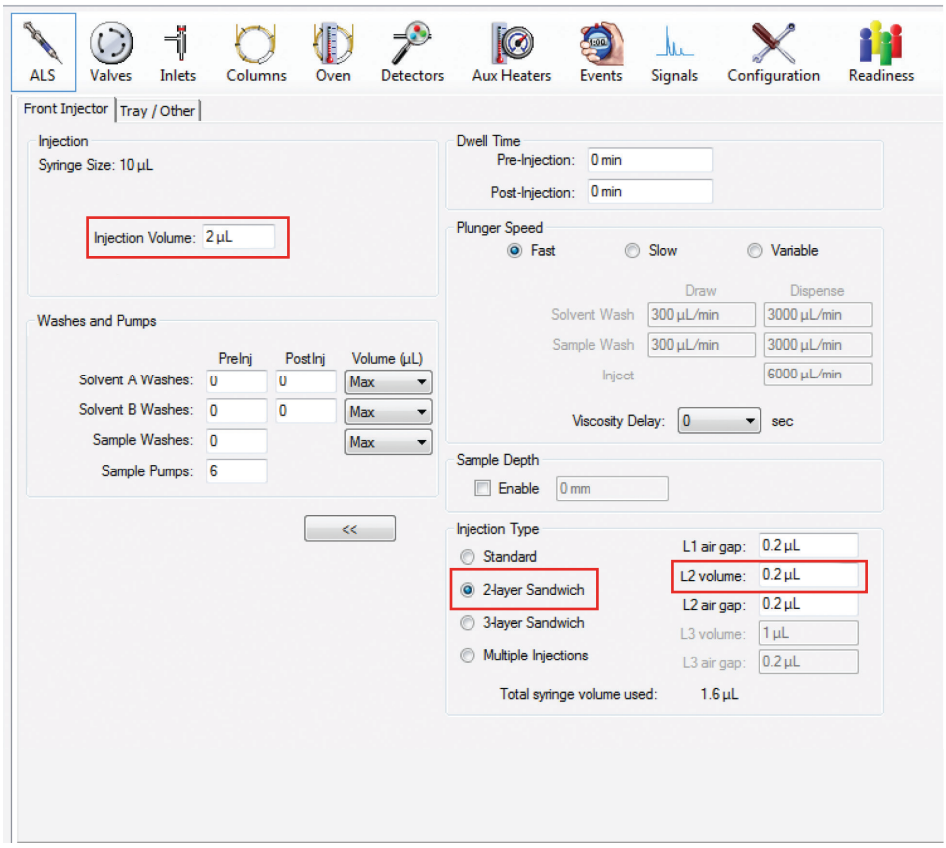

Figure 2. Two-layer sandwich injection setup.

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