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RESEARCH ARTICLE

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Fast pesticide analysis using low-pressure gas chromatography paired with a triple quadrupole mass spectrometer equipped with short collision cell technology

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Rationale: A proof of concept showing GC–MS/MS analysis time for pesticides can be dramatically reduced while maintaining a similar separation efficiency by combining a low-pressure gas chromatography (LPGC) column with the enhanced selected reaction monitoring (SRM) switching speed of the short collision cell of a JEOL JMS-TQ4000GC.

Methods: Triple-quadrupole tandem mass spectrometry (standard EI + at 70 eV) was used to measure pesticides eluting from a low-pressure gas chromatograph capillary column. Three transitions for each of 244 pesticide compounds were measured within an 11-min analysis time, and the data were checked to confirm the method's reproducibility and ability to distinguish all three transitions for each pesticide.

Results: All three transitions for all 244 pesticides were detected in the standard mixture at 1X concentration within the 11-min analysis time. Relative standard deviation (RSD) of peak areas was less than 15% for 242 pesticides, and I/Q RSDs were less than 10% for 242 compounds. Retention time RSD over 15 replicates was less than 0.1%.

Conclusions: Results show that analysis time can be markedly decreased using an LPGC column, and that the ability of the short collision cell to distinguish a large number of coeluting peaks makes the two technologies a natural pairing. The effective measurement of pesticides within a short time could benefit any scientists doing pesticide analysis work.

1 | INTRODUCTION

The use of pesticides presents difficult challenges to scientists. New pesticides are being developed to address ever-changing farming conditions and increasing product yields. As new pesticides are discovered, regulatory scientists must determine the toxicity and dangers to human life and the environment, and develop testing protocols to measure each pesticide. Scientists in commercial or regulatory analytical labs use the developed regulatory methods to test various sample types for pesticide content. Scientists in each discipline rely on analytical methods to perform these tests. For pesticide analysis, any effective method needs to be accurate, sensitive, and fast. In this work, a new method of pesticide measurement was tested for these three criteria, with the primary goal of reducing the analysis time.

Current methods of analysis for pesticides typically include a sample cleanup step, separation by gas chromatography (GC) or liquid chromatography (LC), and then measurement by a detector.[1] In the

case of GC analysis, there are a variety of different detectors available, including the electrolytic conductivity detector (ELCD),[2] halogen specific detector (XSD™),[3] electron capture detector (ECD),[4] and flame photometric detector (FPD).[4] Gas chromatography combined with mass spectrometry (GC–MS) is widely used due to the type of information it provides and its ability to measure complex mixtures. Triple-quadrupole mass spectrometry is a robust analytical technique that features tandem MS (MS/MS) capabilities,[5] which can provide identifying information, and exceptional sensitivity and selectivity. Many regulatory and analytical testing laboratories are using either GC- or LC–MS/MS techniques (or both) for routine pesticide analysis.[6–9] Chlorinated pesticides tend to perform better using GC techniques,[10] whereas highly polar and non-volatile pesticides are better suited to LC separation.[11] Many pesticides overlap both techniques, but if the pesticides of interest are GC-amenable, GC is cheaper to operate and less complex (no mobile phase gradients).

Even though GC–MS/MS can provide exceptional pesticide measurement capabilities, one of the biggest hurdles is that to separate complex mixtures effectively, conventional GC capillary columns are narrower and require longer analysis times. Although longer analysis times provide better separation, analytical and regulatory laboratories can be tasked with running hundreds of samples per day. Under these circumstances, multiple instruments running consecutive analyses would be needed to complete the required testing effectively. Shorter GC times with effective separation capabilities are always needed to increase throughput and reduce cost.

Low-pressure gas chromatography (LPGC) is a technique that uses a short, wide-bore analytical GC column and the vacuum inlet of an MS to significantly decrease elution time with very little sacrifice to separation efficiency.[12] There are two excellent reviews detailing the history, theory, and recent advancements in commercial LPGC viability.[13,14] Although much of this information is beyond the scope of this article, these reviews are highly recommended for an in-depth understanding of how LPGC works. To summarize the theory of LPGC, the wide-bore column and MS vacuum reduce the pressure within the column, which decreases carrier gas viscosity and increases the optimum linear velocity. The result is decreased elution time while maintaining a similar theoretical plate height (separation efficiency). The addition of a restrictor column allows the GC inlet to maintain head pressure and the software to calculate gas flow conditions correctly.[15,16]

Because the pesticide compounds are expected to elute faster and closer together when using an LPGC column, MS/MS will be even more critical to differentiate individual pesticides. For triple-quadrupole MS/MS, dwell time and maximum selected reaction monitoring (SRM) switching speeds are important for either maximizing sensitivity, or measuring more compounds simultaneously. The JEOL JMS-TQ4000GC contains a short collision cell that allows a minimum dwell time of 1 ms and a maximum switching speed of 1,000 SRMs/s. The short collision cell design uses two patented technologies to increase the speed and sensitivity of the analysis. First, pulsed ion accumulation reduces ion loss to maximize sensitivity.[17] The use of a short collision cell allows accumulated ions to be ejected more quickly and completely, which reduces interferences and increases selectivity. The second technology describes a means to control the axial kinetic energy of ions passing through the quadrupole mass filters, so that the transit time is independent of m/z.[18] This permits the use of precise timing to keep the detector turned off except during the time when the ion packets arrive at the detector, resulting in reduced noise and increased sensitivity. The JMS-TQ4000GC also features a differential turbomolecular pump (TMP) capable of pumping 200 L/s at both the analyzer and the ion source. This high pumping capacity provides additional benefits for LPGC column use by further reducing the pressure within the column.

In this study, the separation efficiency and decreased elution time of the LPGC column are combined with the enhanced SRM performance offered by the short collision cell of the JMS-TQ4000GC to measure 244 compounds in a pesticide standard solution mixture in a short analysis time.

2 | METHODS

2.1 | Materials and chemicals

Acetonitrile (HPLC-grade) used for standard sample dilution was purchased from Fisher Scientific (Hampton, NH, USA). Pesticide standard samples were purchased from Restek Corporation (Bellefonte, PA, USA) and AccuStandard, Inc. (New Haven, CT, USA). A full list of pesticides measured in this study are found in Table S1 (supporting information). The final pesticide mixture for testing was created by combining the following standard pesticide mixtures: all nine mixtures from the GC Multiresidue Pesticide Kit (Restek, PN: 32562), Oregon Pesticide Standards #2 and #4 (Restek, PNs: 32587 and 32589), California Pesticide Standards #1 and #4 (Restek, PNs: 34124 and 34127), and Pesticide Mix #22 (AccuStandard, PN: AE-00052). The final target concentration for most compounds was 1 ppm; however, due to the presence of the same pesticides in some standard solutions, 21 of the compounds listed in Table S1 (supporting information) have a concentration of 2 ppm. This standard mixture will be referred to as the 1X standard mixture. For I/Q verification, the 1X standard mixture was also made at double (2X) and at 1/10th (0.1X) the concentration. Grade 4.7 helium for GC carrier gas and liquid nitrogen (LN_2) were purchased from Middlesex Gases (Everett, MA, USA). Boil-off nitrogen from the LN_2 tank was used as a collision gas for MS/MS analysis. A Restek LPGC column kit (PN: 11800), GC inlet liners (PN: 23303), septa (PN: 23865), and caps and vials (PNs: 24385 and 21175) were used for all experiments.

2.2 | Instrumentation

A JEOL JMS-TQ4000GC triple-quadrupole GC–MS/MS equipped with an Agilent 7890B GC was used for all measurements. This

system features a new patented small collision cell optimized for switching speeds of up to 1,000 SRM/s, and allows a minimum dwell time of 1 ms. Experimental operation parameters can be found in Table 1. Transition groupings and dwell times were calculated automatically using the peak-dependent SRM feature in msPrimo™ software, which sets the dwell time to the maximum allowable based on the total number of transitions for each group.

2.3 | SRM transition optimization

Transitions were optimized using built-in software tools. First, five precursor-ions were selected from peaks measured in single-quadrupole mode. Product-ion scans were done for each precursor ion at collisions energies from 5 to 40 eV at 5 eV increments. Collision energies over 40 eV were not evaluated, as almost all collisions at 40 eV were less sensitive than lower-energy collisions. Example product-ion scan results can be found in Figure S1 (supporting information). The five product ions with the greatest peak areas were chosen as SRM transitions, and then confirmed by MS/MS analysis of the 1X standard pesticide mixture. Peak areas of the five transitions were compared, and the two transitions with the lowest peak areas were removed for each analyte for the final experiments. In some cases, transitions with high peak areas in single-compound analysis were obscured by interference ions from other pesticides when measuring a mixture, particularly for low *m/z* transitions. In these cases, higher *m/z* precursor ions were selected for product ion scanning, even if their relative peak intensity was low compared to other precursor ions. More effective SRM transitions were found for some pesticides based on the selectivity of the transition instead of the most sensitive transition based on peak areas from single-quad data.

2.4 | Experiment design

Regulatory compliance testing of pesticides using triple-quadrupole GC–MS/MS typically requires the measurement of a quantitative ion (Q) and one or more qualifier ions (I).[6] Qualifier ions serve to validate that quantitative ion peaks are the target analyte, and not interference ions. Target analytes are verified by matching retention times with qualifier ions and comparing the qualifier ion/quantitative ion ratio (I/Q ratio) to the I/Q ratios measured in a standard solution. In this study, 15 replicates of the 1X standard mixture were measured to calculate the variance of peak area, I/Q ratios, and retention times to assess the accuracy and reliability of the technique. Analysis of 2X and 0.1X standard mixtures was used to compare the stability of I/Q ratios established using 1X data.

3 | RESULTS AND DISCUSSION

3.1 | Results of 1X standard mixture analysis

The total-ion-current chromatograms (TICC) for the 1X standard mixture using LPGC and a standard 30 m column (operation parameters shown in Table S1 [supporting information]) are shown in Figure 1. All compounds measured using LPGC eluted in less than 11 min, and 98% in less than 9 min. These analysis times are approximately 50% faster than our previously optimized method on a traditional 30 m column, and are on par with other studies using LPGC-MS/MS[8,9] and with the manufacturer's reported performance.[19] The TICC and total SRM chromatograms are shown in Figure 2, and selected SRM chromatograms chosen from sections of the TICC with high numbers of coeluting compounds are shown in Figure 3. All three SRM transitions from all 244 compounds were

TABLE 1 Operation parameters for the JMS-TQ4000GC triple-quadrupole GC–MS/MS

Agilent 7890B GC		**JMS-TQ4000GC MS**	
Column	Restek LPGC kit (PN:11800)	Ion source temp.	250°C
Restrictor column	5 m, 0.18 mm i.d.	Interface temp.	290°C
Analytical column	15 m, 0.53 mm i.d., 1 μm Rtx-5 ms	Ionization mode	EI+
Inlet liner	Topaz 4 mm single taper	Ionization energy	70eV
	w/wool on bottom (PN:23303)	Ionization current	100 μA
Inlet temp.	250°C	Measurement mode	SRM, high speed
Carrier gas type	He	SRM grouping	Peak-dependent
Flow rate	1.000 mL/min	Target cycle time	Approx. 240 ms
Injection mode	Pulsed splitless	Acquisition rate	Approx. 4 Hz
Pulsed pressure, time	206.84 kPa, 0.550 min	Precursor window	0.8 u
Purge flow, time	50 mL/min, 1.0 min	Relative EM voltage	500 V
Septum purge flow	3.0 mL/min	Collision gas	N_2, 10%
Saver flow, time	15 mL/min, 5.0 min	**Oven program**	
Injection volume	1.0 μL	70 °C (1 min) →	
Total runtime	11 min	35°C/min → 320°C (3 min)	

Note: SRM, selected reaction monitoring.

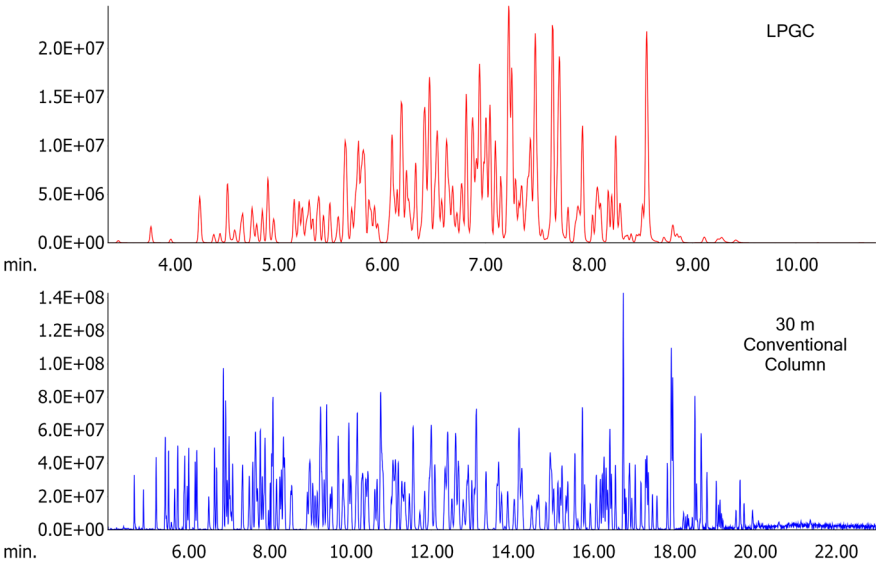

FIGURE 1 Total-ion-current chromatogram (TICC) of the 1X standard mixture measured with LPGC (top) and a traditional 30 m GC column (bottom) [Color figure can be viewed at wileyonlinelibrary.com]

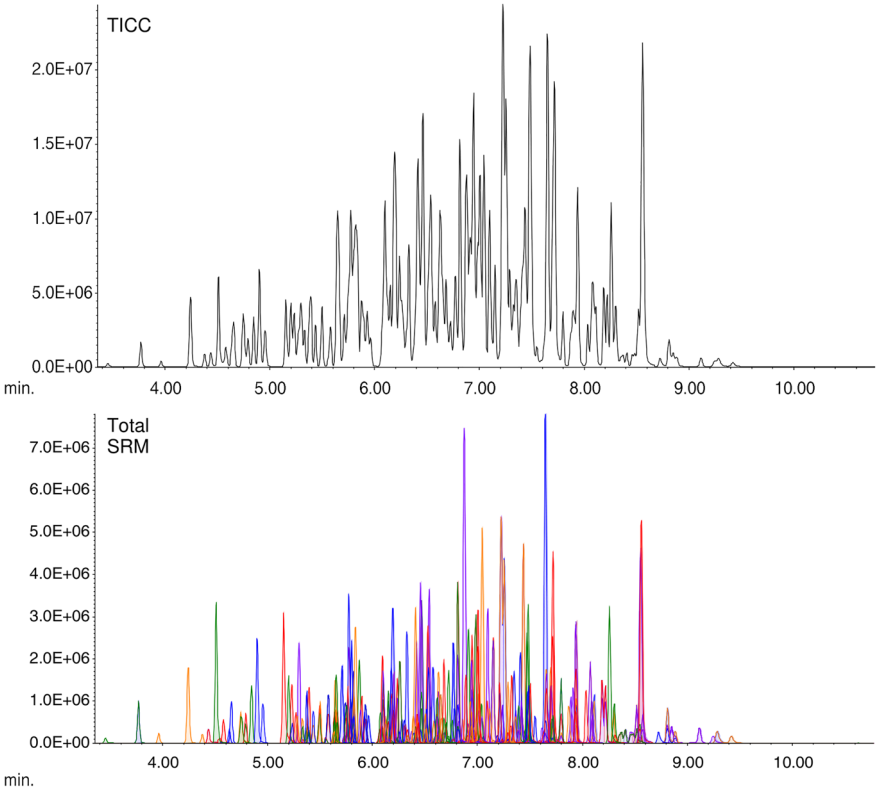

FIGURE 2 Total-ion-current chromatogram (top) and total selected reaction monitoring (SRM) transition chromatogram (bottom) of the 1X standard mixture [Color figure can be viewed at wileyonlinelibrary.com]

observed in their respective SRM chromatograms at a signal:noise of at least 10:1. Even though TICC chromatographic resolution may not be equivalent to that of a traditional 30 m GC column, the SRM chromatograms demonstrate that the LPGC column still separated the compounds effectively enough for the mass spectrometer to measure them in MS/MS mode, while completing the overall measurement in a much shorter timeframe.

In general, excellent SRM chromatographic resolution was observed for most compounds as seen in the HCH and chlordane isomer SRM chromatograms (Figure 3). For a few compounds, such as cyfluthrin, cypermethrin, and DDT isomers (Figure 4), LPGC separation was not effective enough to observe an 80% valley; however, isomers could still be observed in most cases, and I/Q's at other concentrations were matched within 30% (RSD data are listed in Table S1 [supporting information]). The short collision cell was able to measure all pesticides at 1X concentrations, even during time periods when compound coelution was maximized. With the capability of 1,000 SRMs/s, analysis can be configured to maximize sensitivity with fewer total compounds, or maximize the number of compounds that can be analyzed in a single analysis. Results also

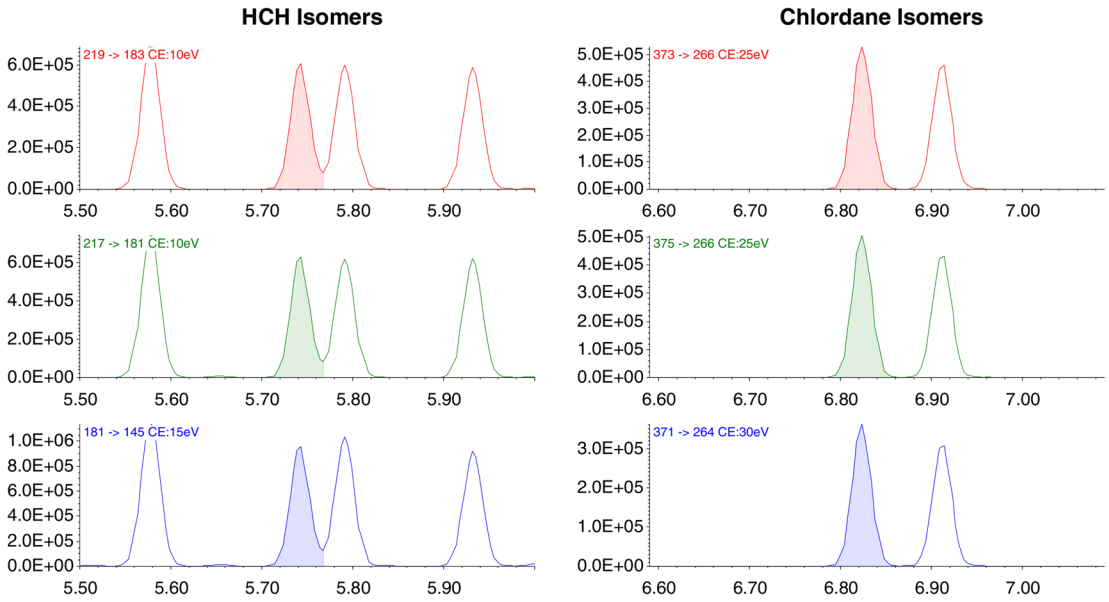

FIGURE 3 Selected reaction monitoring (SRM) chromatograms for HCH and chlordane isomers [Color figure can be viewed at wileyonlinelibrary.com]

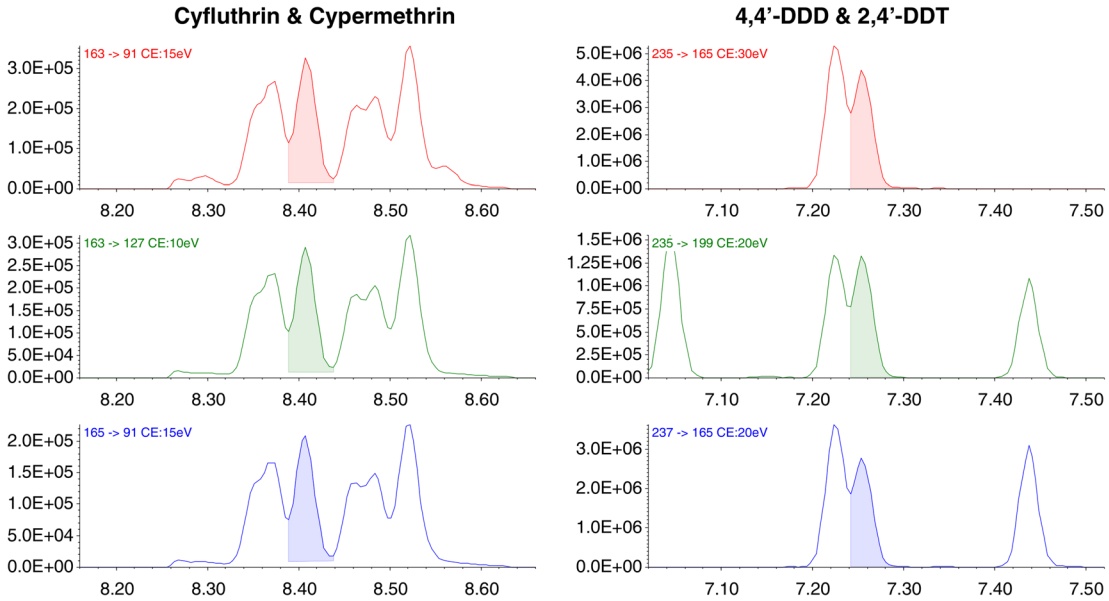

FIGURE 4 Selected reaction monitoring (SRM) chromatograms for cyfluthrin, cypermethrin, 4,4'-DDD, and 2,4'-DDT [Color figure can be viewed at wileyonlinelibrary.com]

demonstrate how the selectivity of MS/MS is a key factor to distinguishing so many pesticides in a complex mixture.

Area, retention time, and I/Q RSDs were calculated for 15 replicates of the 1X mixture (Table S2 [supporting information]). Only 2 compounds had area RSDs over 15% (chlorantraniliprole and clofentezine), and 236 compounds had RSDs less than 5%. Similar results were observed for I/Q RSDs: 236 compounds had RSDs less than 5%, and only 2 compounds had RSDs greater than 10%. Retention time RSD was less than 0.1% for all compounds, indicating excellent retention time reproducibility over several injections. In general, statistical analysis over 15 replicates shows that the technique is robust and suitable for routine analysis.

3.2 | Results of the 2X and 0.1X standard mixtures

An overview of the I/Q comparison results for the 2X and 0.1X standard mixtures against the 1X standard mixture is presented in Table S1 (supporting information). Both I/Q ratios of all pesticides measured in the 2X standard mixture matched the 1X standard

mixture within ±30%. For the 0.1X standard mixture, peak intensities for 7 pesticides (acetamiprid, captafol, captan, chlorthiophos III, dichlofluanid, folpet, and tolyfluanid) fell below the limit of quantitation. One qualifying ion for both linuron and fipronil, and both qualifying ions for fenoxycarb, failed to be within ±30% of the average I/Q obtained from the 1X measurement. Both qualifying ions for all other 234 pesticides in the 0.1X standard mixture were measured within ±30% of the 1X values. Values for I/Qs were generally consistent across 1X, 2X, and 0.1X standard mixtures, indicating that LPGC measurement on a JMS-TQ4000GC is capable of routine analysis for regulatory testing of pesticides. Qualifying ion I/Qs that did not match within ±30% of the 1X reference data may be the result of interfering ions having more influence at lower concentrations. This could be addressed by selecting different SRM transitions. Although this study was not focused on limits of detection (LODs), 0.1X concentrations are sufficient for measuring many of the regulated pesticides listed in the U.S. Code of Federal Regulations and European Union Regulation (EC) No 396/2005.[20,21] Future studies would benefit from incorporating LOD, recovery, and matrix effect experiments.

4 | CONCLUSION

All 244 pesticides eluted from the GC column in less than 11 min, demonstrating the viability of using LPGC to separate a large number of pesticides effectively. An approximately 50% decrease in analysis time compared to a traditional 30 m column was recorded, and all pesticides and confirmation ions were observed in their corresponding SRM chromatograms for the 1X and 2X standard mixtures. The short collision cell in the JMS-TQ4000GC provided the necessary switching speeds needed to elucidate individual pesticide peaks from a complex TICC. Low RSDs for peak area, I/Q, and retention time over 15 replicates indicate a stable and reproducible method, which could be applied to pesticide analysis for a variety of applications. The short collision cell technology combined with LPGC separation could be used as a shotgun screening approach to pesticide analysis, or if there are fewer compounds that need to be monitored, current routine pesticide analyses could be sped up significantly without sacrificing sensitivity.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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