Application Note

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Impact of GC Liners on Lab Productivity While Analyzing Complex Matrices

Evaluation of liner lifetime for GC/MS analysis of pesticides in Traditional Chinese Medicine matrices

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Abstract

This application note summarizes the performance comparison of three popular gas chromatograph inlet liners for the analysis of pesticides from traditional Chinese medicine (TCM). A total of 35 pesticides were chosen for the study in Danshen root (*Salvia miltiorrhiza*) matrix. A QuEChERS sample preparation method was used to extract target analytes from the matrix. An Agilent 8890 GC using a midcolumn backflush configuration, coupled with an Agilent 7000D triple quadrupole mass spectrometer (GC/TQ), was used for the analysis. The analytical performance of the Agilent Ultra Inert splitless single taper liner with glass wool, the Agilent Ultra Inert splitless dimpled 2 mm id liner, and the Agilent Ultra Inert splitless single taper liner with frit was examined using the average target response and recovery to identify the best choice of liner for enhanced lab productivity in the trace analysis of complex TCM matrices such as Danshen root.

Introduction

TCM is a medical system that has been in use for over 2,000 years. Due to widespread use, the Chinese Pharmacopoeia has released guidance for the detection and regulation of pesticides in TCM substances.[1] Within TCM, teas, powders, and extracts are taken from plants, including the leaves, seeds, roots, stems, and bark. Among the different matrix types analyzed in TCM, roots such as Danshen root tend to be the most complex, and can cause complications in pesticide detection. Previous GC/TQ analyses have shown promise for monitoring pesticides in TCM matrices, but optimization of those methods could be improved by reducing matrix transfer.[2]

The GC inlet liner functions as the interface between the sample injector and the analytical column. Maintained at high temperature, the GC inlet liner transforms the injected liquid phase sample into the gaseous phase, creating a homogenous blend of sample and carrier gas. The liner then transfers the sample to the column head as a tight band. Liners with a deactivated barrier, such as glass wool or glass frit, provide extended surface area for rapid and uniform vaporization of the sample. They also act as a barrier to protect the GC column and MS source from complex matrix residues. Based on the physical and chemical complexities of the matrix, targets, and injection techniques, a wide choice of liners is available.

A splitless injection mode, where the split vent is closed to transfer most of the sample to the analytical column, is a popular approach for trace analysis. With splitless injection mode, the liner is prone to greater contamination from matrix components, and replacement is required more frequently for optimum instrument performance. A liner that maximizes the number of injections without compromising the analytical performance is valuable to minimize instrument downtime and maximize lab productivity. This application note compares the performance of three Agilent liners for the analysis of pesticides in a complex TCM matrix. The performance of each liner type was evaluated by monitoring the response and recovery of 35 target pesticides with repeated splitless injections of matrix-spiked samples.

Experimental

Chemicals and reagents

A custom mixed standard of 35 target pesticides and an internal standard (ISTD) of triphenyl phosphate, both at concentrations of 500 µg/mL, were purchased from AccuStandard (New Haven, CT, United States). Both standards and ISTD were diluted using acetonitrile to prepare the working standard solutions. When not in use, all standards were stored as per the manufacturer's recommendation either at 5 °C or –20 °C. LC/MS grade acetonitrile was procured from Agilent. Acetic acid for sample preparation was purchased from MilliporeSigma (Burlington, MA, USA).

Sample extraction

Danshen root matrix was obtained from a local TCM pharmacy. The extraction protocol followed the procedure described in the Chinese Pharmacopoeia method No. 5 (Figure 1).[1] Briefly, the sample was homogenized using a blender. A 3 ±0.1 g aliquot was weighed into a 50 mL conical polypropylene tube (part number 5610-2049). Fifteen milliliters of 1% v:v acetic acid solution was added to each tube. The tube was then vortexed for 1 minute and incubated at room temperature for 30 minutes. An additional 15 mL of acetonitrile was added to each tube along with two ceramic homogenizers (part number 5982-9313). The sample was further extracted in a mechanical shaker at 750 rpm for 5 minutes. Then, the Agilent QuEChERS extraction kit containing 6 g of $MgSO_4$ and 1.5 g of sodium acetate (part number 5982-5755) was added to the 50 mL tube. The sample was again placed into the mechanical shaker at 750 rpm for 3 minutes. After extraction, the sample was cooled in an ice bath for 10 minutes, followed by centrifugation at 4,000 rpm for 5 minutes at 10 °C. A 9 mL aliquot of the supernatant was then transferred to the dispersive solid phase extraction tube (part number 5982-5156) containing 90 mg of graphite carbon black (part number 64100G) and vortexed for 1 minute. Then, the sample was extracted with the mechanical shaker at 750 rpm for 5 minutes. The sample was then centrifuged at 4,000 rpm for 5 minutes at 10 °C. A 5 mL aliquot of the supernatant was transferred to a test tube and blown to dryness under a gentle stream of nitrogen. The dry sample residue was reconstituted using 1 mL of acetonitrile and centrifuged at 4,000 rpm for 7 minutes at 10 °C. The supernatant was transferred to an Agilent 1.5 mL high recovery vial (part number 5183-2073) for analysis. A summary of the sample extraction consumables is listed in Table A1 in the Appendix.

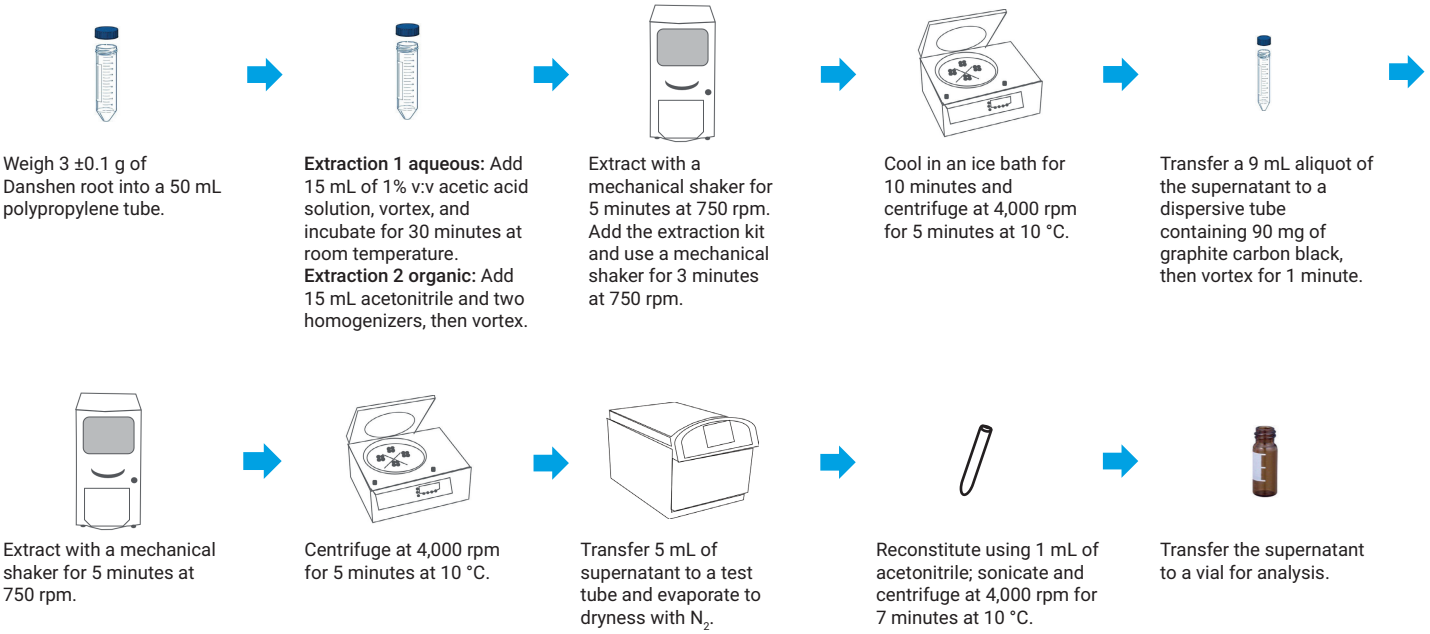


Figure 1. QuEChERS sample preparation protocol of Danshen root.

Instrumentation

Analysis was performed on a 8890 GC coupled with a 7000D triple quadrupole MS system. Midcolumn backflush was configured (union kit part number G1472A and PSD module part number G3559A) on the GC instrument to facilitate separation and concurrent backflush using two sequentially connected Agilent J&W DB-17, 15 m × 0.25 mm, 0.25 µm analytical columns. The Agilent 7693A GC automatic liquid sampler was fitted with an Agilent Blue Line 10 µL polytetrafluoroethylene (PTFE)-tip plunger tapered syringe (part number G4513-80203). Three liner styles with different barrier styles were tested for splitless injections in this study, which are listed in Table 1.

Table 1. Liner styles and shortened names to be used in the text.

Liner Description	Part Number	Name Used in Text
Agilent Ultra Inert Splitless, single taper with glass wool	5190-2293	Wool liner
Agilent Ultra Inert Splitless, dimpled, 2 mm id	5190-2297	Dimpled liner
Agilent Ultra Inert Splitless, single taper with frit	5190-5112	Fritted liner

The GC conditions are listed in Table 2, and MS source parameters are included in Table 3. Multiple reaction monitoring (MRM) mode was used for data collection, and the transitions were selected based on Chinese Pharmacopoeia recommendations (Table A2).[1] Retention time shifting was avoided by using the retention time locking (RTL) software tool; RTL has been described in previous publications.[3] A neat standard mix of the pesticides at 100 µg/L was used to lock the retention time of the target analyte Isocarbophos (MRM 135.7 → 108.0) to 14.05 minutes. Data were acquired on Agilent MassHunter Workstation GC/MS Data Acquisition software (version 10.1.49) and processed using MassHunter Quantitative Analysis software (version 10.2).

Table 2. Agilent 8890 GC parameters.

Parameter	Value
Injection Volume	1 µL
Inlet	Multimode in pulsed splitless mode, 265 °C
Inlet Septum	Nonstick bleed temperature optimized 11 mm inlet septa (part number 8010-0223)
Injection Pulse Pressure	30 psi until 0.5 min
Purge Flow to Split Vent	60 mL/min at 0.5 min
Septum Purge Flow	3 mL/min
Carrier Gas	Helium
Columns	Two Agilent J&W DB-17, 15 m × 0.25 mm, 0.25 µm (part number 122-4712)
Column Flow Rate	Column 1: 1.2 mL/min Column 2: 1.5 mL/min Backflush at 23.5 min on column 1 with –1 mL/min to 26.75 min (column ramp rate 100 mL/min)
Column Flow Rate During Postrun	4.15 mL/min for column 1 and 4.57 mL/min for column 2 for 4 min
Oven Temperature Program	Initial: 80 °C (hold: 1 min) – Ramp 1: 40 °C /min to 200 °C – Ramp 2: 2 °C /min to 230 °C – Ramp 3: 40 °C /min to 300 °C (hold: 6 min) Postrun: 4 min

Table 3. Agilent 7000D triple quadrupole configuration and parameters.

Parameter	Value
Transfer Line	260 °C
Ionization	Electron Ionization (EI)
Mode	MRM
Source Temperature	230 °C
Quad Temperature	150 °C
Solvent Delay	6 min
Gain Factor	10

Matrix-matched calibration

Pooled matrix blank extractions were used to provide sufficient sample volume for the preparation of matrix-matched calibration levels. A five-point postextraction matrix-matched calibration curve (5, 10, 50, 100, and 200 µg/L) was prepared from the matrix blank by spiking appropriate concentrations of the working standard. The ISTD concentration was maintained at 50 µg/L at all calibration levels. For every liner type (Table 1), MRM data of the matrix blank and matrix-matched calibrators were collected to construct calibration curves with ISTD correction.

Evaluation of liners

Danshen root matrix was spiked before extraction with 75 µg/L of target analytes and subjected to the sample preparation protocol (Figure 1) to prepare matrix-spiked samples for analysis of each liner type (Table 1). Similar to matrix-matched calibration levels, the ISTD concentration was also maintained at 50 µg/L for matrix-spiked samples. Matrix-spiked samples were injected continuously to monitor the absolute response and recovery values of each target analyte. The recovery value for each target was calculated using the matrix-matched calibration curve equations generated for each liner type. The calibration curve constructed using the first liner of each liner type was used to calculate recovery values. Deviations in response and recovery values for replicate injections were monitored for the failure of acceptable performance criteria. Acceptable performance criteria for each liner type were considered to be 70% of target analytes with an area deviation ±20% relative standard deviation (RSD) and 60% of target analytes with recovery values between 70 and 130%.

Upon failure of acceptable performance criteria, the column was trimmed (20 cm), and inlet maintenance was completed. The maintenance consisted of changing the septum and liner after cleaning the multimode inlet with isopropyl alcohol-wetted swabs. Then, the instrument was quick-tuned, and the method was relocked with one RTL run to restore instrument performance comparable to the initial condition. If results remained unacceptable after the liner replacement and preventative maintenance steps, then column 1 (which connected between the inlet and purged Ultimate union) was replaced.

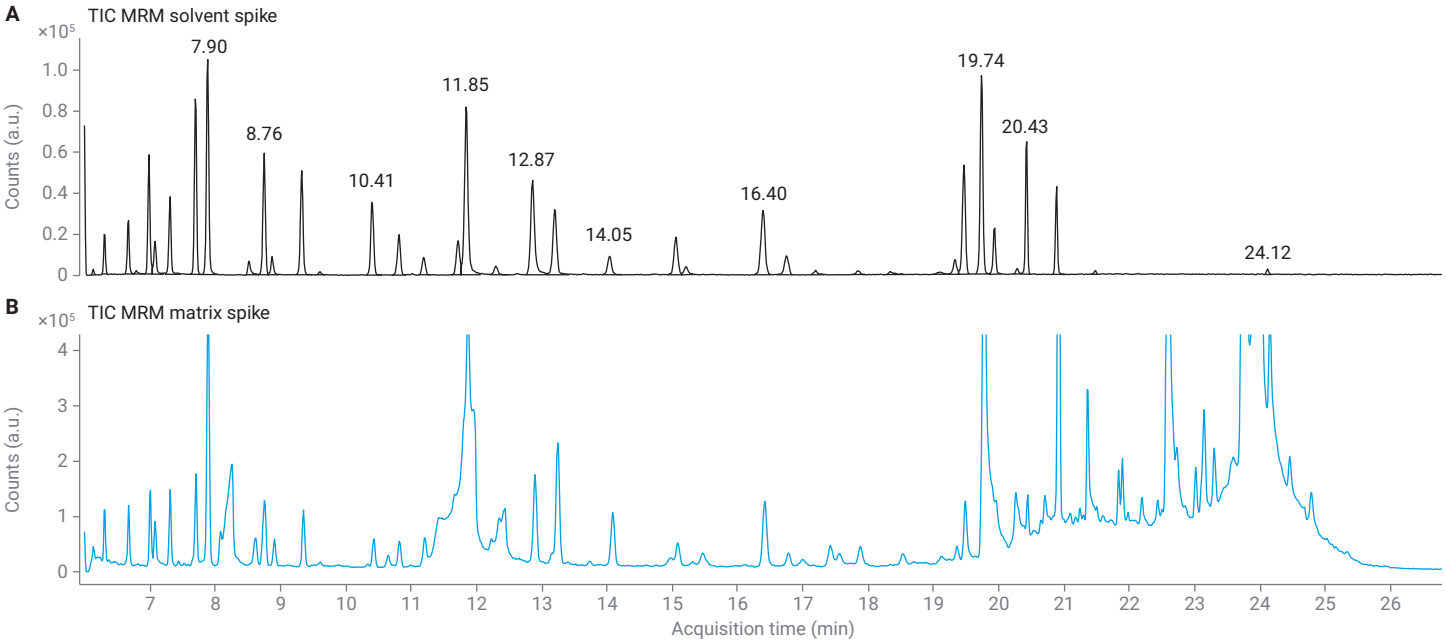
For each liner type, the experiment was repeated with a minimum of three fresh liners, and the average response and recovery values were used to assess the liner performance. For the dimpled liner, in addition to the default injection volume of 1.0 µL, data were also collected with an adjusted injection volume of 0.3 µL to examine performance at a lower vapor volume. The 0.3 µL injection was performed using Agilent Blue Line 1 µL GC autosampler syringe (part number G4513-80215). The 1.0 µL injection expands to 138% capacity of the dimpled liner, as calculated by the vapor volume calculator. This volume was tested as a direct comparison to the other liners, as absolute response would be different when using a lower injection volume. The adjusted volume of 0.3 µL was chosen because the injection expands to just 41% capacity of the dimpled liner with the 30 psi pressure pulse. Performance between both injection volumes on the dimpled liner was similar; therefore, for the present study, the 1.0 µL injection was used for evaluation.

With the continued injection of complex matrix samples, high boiling residues can deposit in the liner and analytical column head, resulting in poor peak shape, reduced response, and peak mislabeling. To avoid this challenge, the GC column was trimmed with every liner change. Due

to this action, the column length was altered with every liner change and potentially resulted in target retention time shifts and peak mislabeling, which was avoided with the RTL procedure described.

Results and discussion

The target analytes with similar MRM transitions, like fipronil and fipronil sulfide and dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD), are well separated using the described chromatographic method of 25 minutes. While a longer 51-minute method is highlighted in the Chinese Pharmacopeia guidelines, isomer separations such as o-demeton and s-demeton are adequate with the 25-minute method. The shorter duration further supports previous work using an Agilent Intuvo 9000 GC and a 7000D triple quadrupole mass spectrometer.[2] In addition, RTL ensured unambiguous identification of the target peaks, even if there was a matrix impact in target elution time. All matrix-matched calibration curves used in this study were linear with an R^2 ≥0.995.



No.	Name	RT
1	O-Demeton	6.32
2	Ethoprophos	6.67
3	Chlordimeform	7.00
4	Sulfotep	7.08
5	Phorate	7.32
6	Alpha BHC	7.70
7	Terbufos	7.88
8	S-Demeton	7.90
9	Monocrotophos	8.52

No.	Name	RT
10	Gama BHC	8.76
11	Fipronil desulfinyl	8.87
12	Beta BHC	9.34
13	Delta BHC	10.41
14	Aldrin	10.82
15	Parathion-methyl	11.19
16	Fipronil sulfide	11.72
17	Fipronil	11.83
18	o,p'-Dicofol	11.85

No.	Name	RT
19	Parathion	12.31
20	p,p'-Dicofol	12.87
21	Isofenphos-methyl	13.20
22	Isocarbophos	14.05
23	Endosulfan I (alpha isomer)	15.06
24	Fipronil sulfone	15.20
25	p,p'-DDE	16.40
26	Dieldrin	16.75

No.	Name	RT
27	Fenamiphos	17.46
28	Phosfolan-methyl	18.35
29	Nitrofen	19.34
30	o,p'-DDT	19.47
31	p,p'-DDD	19.74
32	Endosulfan II (beta isomer)	19.94
33	p,p'-DDT	20.43
34	Endosulfan sulfate	20.89
35	Coumaphos	24.12

Figure 2. Total ion chromatogram (TIC) MRM trace of 35 pesticides demonstrating the elution profile (A: 200 µg/L spiked in acetonitrile; B: 200 µg/L spiked in the matrix). The retention time details of all 35 target analytes are included as an inset table.

Sample preparation extraction efficiency

The QuEChERS sample preparation protocol provided efficient extraction of most of the target pesticides. A few targets like chlordimeform, fenamiphos, phorate, and terbufos exhibited reduced recovery within a range of 30 to 70%, while o- and s-demeton exhibited recovery of <30% using the QuEChERS sample preparation protocol.

Figure 2 shows the difference between a sample injected in solvent compared to extracted matrix. Acceptable performance criteria for each liner type were considered to be 60% of target analytes with recovery values between 70 and 130%. All liner types met the acceptable criteria, with 80% of the analytes recovered in the wool liner, 71% recovered in the dimpled liner, and 83% recovered in the fritted liner with recovery values inside the 70 to 130% recovery range.

Liner response and recovery reproducibility

Target analyte response was investigated for the wool and fritted liner with 120 matrix-spiked replicate injections, and 85 replicate injections on the dimpled liner (Figure 3). The initial injection resulted in a peak symmetry value of <1.5 for each liner type. With progressive replicate injections, the target analyte response was found to decrease, and peak symmetry values would increase above 1.5, which was considered unacceptable performance. As an example, response reduction and decay in peak symmetry for the dicofol isomers are presented in Figure 4 for each liner type. Peak symmetry was within an acceptable limit of 1.5 for the first 50 injections using the wool liner, for 25 injections using the dimpled liner, and for 75 injections using the fritted liner. The response RSD was within the 20% limit for 45 injections on the wool liner, 30 injections on the dimpled liner, and 70 injections on the fritted liner.

Similar to the observation of response, recovery values decreased over multiple replicate injections (Figure 5). Acceptable recovery was observed for 50 injections on the wool liner, 24 injections on the dimpled liner, and 70 injections on the fritted liner.

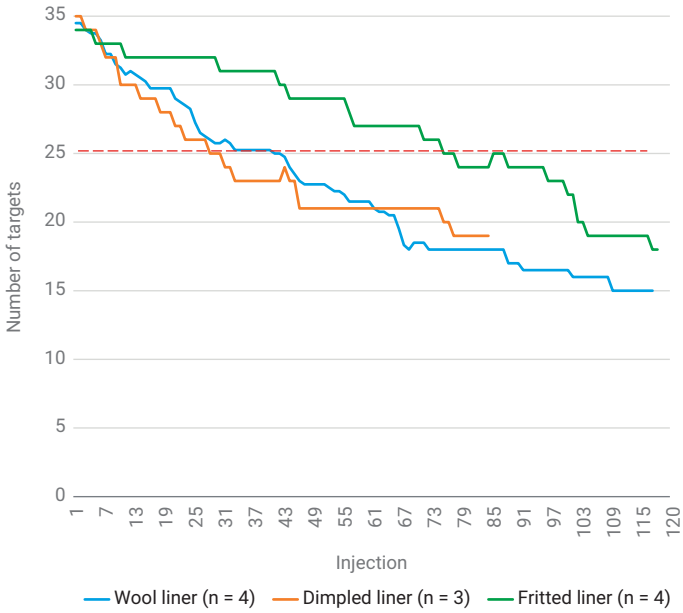


Figure 3. Number of injections possible where at least 26 target analytes meet the 20% RSD acceptability criteria. The red dotted line represents a cutoff where <26 target analytes are meeting the 20% RSD acceptance criteria.

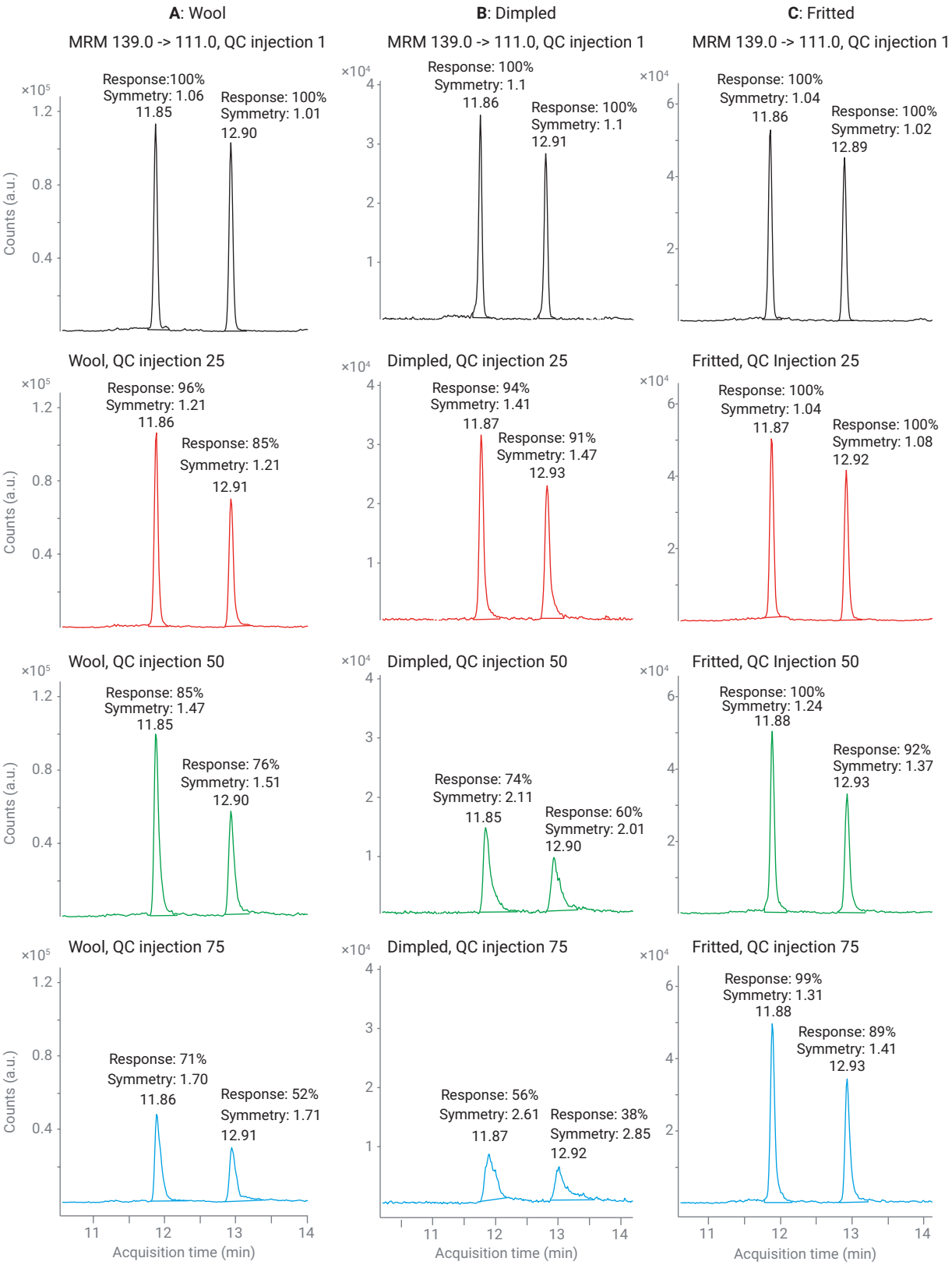


Figure 4. MRM data (139.0 → 111.0) of o,p'-dicofol (retention time: 11.8) and p,p'-dicofol (retention time: 12.9) with injection number 01 (black trace), 25 (red trace), 50 (green trace), and 75 (blue trace) using wool (A-left), dimpled (B-middle) and fritted (C-right) liners. The response reduction percentages and peak symmetry values are included for each MRM window.

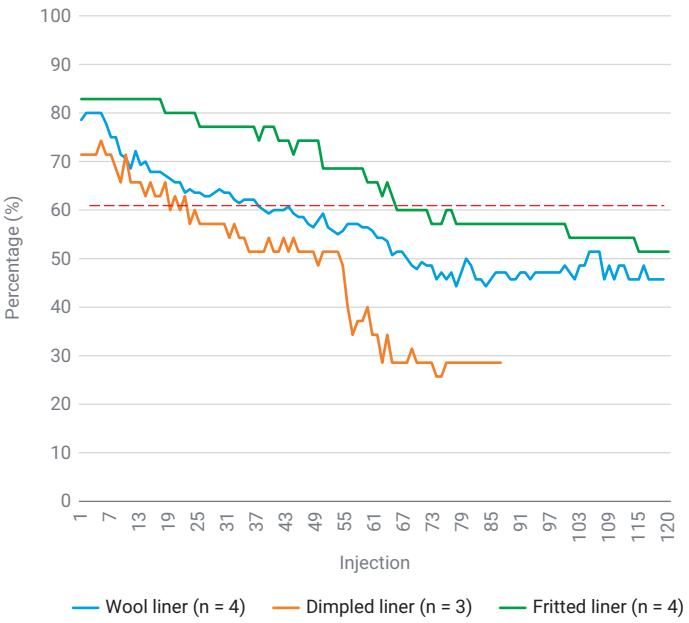


Figure 5. Number of injections possible where at least 60% of target analytes meet the acceptable recovery criteria of 70 to 130%, based on recovery in matrix. The red dotted line represents a cutoff at which the recovery of at least 60% of all target analytes fails to meet the accepted recovery criteria of 70 to 130%.

The average lifetimes of all three liner types are calculated and summarized in Figure 6. The fritted liner was observed to outperform other liner types for the analysis of pesticides from Danshen root with an average lifetime of 70 injections. The fritted glass barrier helps to prevent matrix residues from entering the column head, resulting in an extended column lifetime.

Additionally, it was observed that for a fixed injection volume of 1 µL, the dimpled liner transferred more matrix residue onto the column with repeated runs, which required more frequent column replacement compared to the other two liners. Response data from the fritted liner demonstrated that individual compounds react differently to liner aging. However, when a new liner is installed and the preventative maintenance steps are performed as detailed before, the instrument performance is restored to initial conditions in most cases (Figure 7).

The response of p,p'-DDD was stable, and performance remained acceptable over 100 injections. p,p'-Dicofol shows a slowly reducing response, which is restored with each liner change. Phosfolan-methyl response was found to be very sensitive to liner aging, where response degradation happens quickly along each replicate injection. However, similar to p,p'-dicofol, phosfolan-methyl response was restored to initial results after a liner change. Unlike the other target analyte examples, phorate response is continuously reducing over 100 replicate injections, and a liner replacement does not restore signal strength. The observations seen in phorate may be due to other factors beyond just liner performance, such as column health beyond the trimmed section, source cleanliness, or target stability in the studied matrix.

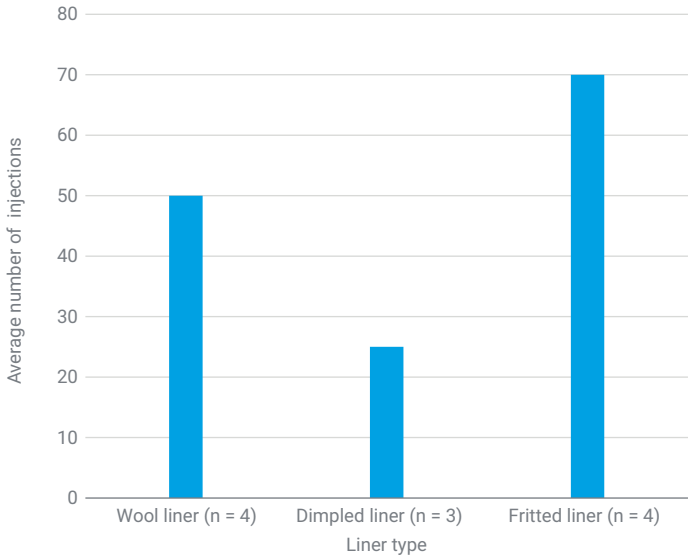


Figure 6. Average number of injections before exceeding liner acceptable performance criteria.

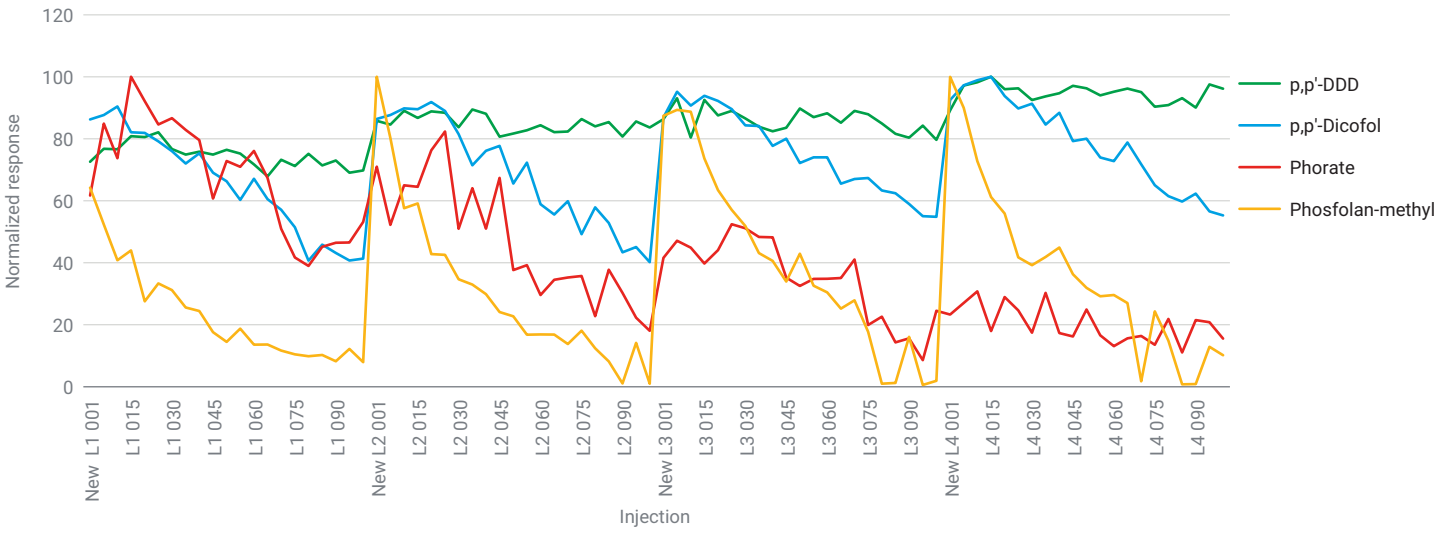


Figure 7. Normalized response of four target analytes over 100 replicate injections of matrix extracted samples using the fritted liner. Liner was replaced every 100 injections for a total of 400 injections.

Conclusion

This application note highlights the importance of GC inlet liner selection for lab productivity and the quality of analytical results when analyzing complex TCM matrices. The choice of liner for routine analysis of complex matrices is especially critical, as it can cause complications in pesticide detection. The results here demonstrated that the Agilent Ultra Inert splitless single taper liner with frit provides the longest lifetime of 70 matrix injections and the most robust results, aiding in the confident analysis of challenging matrices like Danshen root from traditional Chinese medicine. The entire analytical workflow contributes to the lab productivity with confident analytical results. A reliable sample preparation approach similar to the one described here is necessary as well.

References

1. Method No. 5, Multi-Residue Determination Method for Banned Pesticides in Medicinal Materials and Decoction Pieces (Plants), *the Chinese Pharmacopoeia,* **2020**.
2. Zhang, J. Screening of Pesticide Residues in Traditional Chinese Medicine with the Agilent Intuvo 9000 GC, *Agilent Technologies application note*, publication number 5994-4044EN, **2021**.
3. Retention Time Locking with the MSD Productivity ChemStation, *Agilent Technologies*, publication number 5989-8574EN, **2008**.

Appendix

Table A1. Consumables used for sample preparation.

Consumable	Part Number
Agilent QuEChERS Extraction Kit	5982-5755
Dispersive SPE Kit	5982-5156
GCB Bond Elut Carbon Bulk	64100G
Ceramic Homogenizer, 50 mL, 100/pk	5982-9313
50 mL Conical Polypropylene Tube	5610-2049
High Recovery Vials, 1.5 mL, 100/pk	5183-2073
PTFE/red Silicone Bonded Screw Cap 100/pk	5190-7024

Table A2. MRM parameters for target analytes and ISTD (MS1 and MS2 resolution: LowRes).

Compound Name	Precursor Ion	Product Ion	Dwell (ms)	CE (ev)
Aldrin	254.9	220	10	20
Aldrin	262.7	192.7	10	30
Alpha-/Beta-/Gama-/Delta-BHC	181	145	10	15
Alpha-/Beta-/Gama-/Delta-BHC	218.7	182.9	10	5
Chlordimeform	117	90	10	20
Chlordimeform	152	117	10	15
Chlordimeform	196	181	10	5
Coumaphos	361.8	225.8	10	15
Coumaphos	361.8	109	10	15
p,p'-DDE	316	246	10	25
p,p'-DDE	246	176	10	30
o,p'-DDT/p,p'-DDT/p,p'-DDD	235	199	10	15
o,p'-DDT/p,p'-DDT/p,p'-DDD	235	165	10	25
p,p'-DDT/p,p'-DDD	237	165	10	25
o,p'-/ p,p'-Dicofol	139	111	10	15
o,p'-/ p,p'-Dicofol	250	139	10	15
Dieldrin	262.9	193	10	35
Dieldrin	276.8	240.7	10	10
Endosulfan I (alpha isomer)	240.8	205.6	10	15
Endosulfan I (alpha isomer)	194.9	159	10	10
Endosulfan II (beta isomer)	206.8	171.8	10	15
Endosulfan II (beta isomer)	194.8	159	10	10
Endosulfan sulfate	273.8	238.9	10	15
Endosulfan sulfate	271.8	141	10	40
Endosulfan sulfate	271.8	236.7	10	15
Ethoprophos	199.7	157.8	10	5
Ethoprophos	157.8	96.7	10	20
Fenamiphos	217	202.1	10	10
Fenamiphos	303.1	153.9	10	30
Fenamiphos	303.1	122	10	20
Fipronil	367	255	10	25
Fipronil	351	255	10	20
Fipronil	367	213	10	35

Compound Name	Precursor Ion	Product Ion	Dwell (ms)	CE (ev)
Fipronil desulfinyl	388	281	10	35
Fipronil desulfinyl	388	333	10	20
Fipronil sulfide	420	255	10	20
Fipronil sulfide	420	351	10	12
Fipronil sulfone	383	255	10	20
Fipronil sulfone	383	213	10	32
Isocarbophos	120.7	65	10	20
Isocarbophos	135.7	108	10	15
Isofenphos-methyl	199	65	10	40
Isofenphos-methyl	199	121	10	15
Monocrotophos	192	127.1	10	10
Monocrotophos	127	109	10	12
Nitrofen	201.8	138.7	10	28
Nitrofen	282.8	253	10	10
Nitrofen	282.8	201.8	10	15
o-/s-Demeton	88	45	10	25
o-/s-Demeton	88	60	10	4
Parathion	139	109	10	10
Parathion	291	81	10	30
Parathion	291	109	10	25
Parathion-methyl	263.1	79	10	35
Parathion-methyl	263.1	109	10	13
Phorate	260	75	10	5
Phorate	230.8	128.6	10	25
Phosfolan-methyl	167.8	109	10	10
Phosfolan-methyl	91.9	63.8	10	10
Sulfotep	321.8	201.9	10	20
Sulfotep	322	174	10	15
Terbufos	230.9	129	10	25
Terbufos	230.9	175	10	13
Triphenyl phosphate (TPP) ISTD	326	233	10	18
Triphenyl phosphate (TPP) ISTD	326	215	10	25

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