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Analysis of California Residual Solvent Targets in Cannabis Extract

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Introduction

With the gradual legalization of cannabis use in some jurisdictions, regulation and testing is also seeing growth. Cannabis testing generally has two types: microbiological and chemical. Within chemical testing, there are product quality tests that focus on potency and terpenes, and product safety tests that focus on pesticides, heavy metals, and the spotlight of this note, residual solvents.

The United States Pharmacopeia (USP) separates solvents into three different classes.[1] Class 1 solvents are to be avoided. They are often known or suspected carcinogens and/or environmental hazards. Class 2 solvents are to be limited. Their toxicity may not be as serious as Class 1 solvents, but they are still dangerous and can be teratogens, neurotoxins, or nongenotoxic carcinogens. Class 3 solvents have the lowest toxicity potential, and often have no need of a health-based exposure limit.

The California Department of Cannabis Control (DCC) has outlined twenty-one solvents divided into two categories (**Table 1**) that must be tested on cannabis samples. Category 1 solvents have the lowest action limits at 1 ppm, while Category 2 solvents have action limits that can be much higher. It is important to note that the categories that the DCC have outlined do not necessarily coincide with the class system used by the USP.

Residual solvents are measured by GC-MS headspace analysis. In this technique, volatile gas analytes are sampled from the top of a sample vial (the headspace) and introduced directly into the GC. This technique is selective to highly volatile gas analytes and typically results in less complex chromatographic data, which is highly appropriate for samples such as cannabis, that have complex matrices. There are extensive resources on headspace analysis, and because the specifics of the technique are beyond the scope of this application note, the reader is directed to those sources.[2,3]

In this technical note, residual solvents from the California DCC list were measured for standard samples and a cannabis sample using a Zebron ZB-624*PLUS* GC column. Linearity, repeatability, and detection limits were investigated, as well as practical application to cannabis samples.

Sample Preparation

The standard sample used for this study was California Residual Solvents Calibration Rev 1, purchased from Absolute Standards, Inc. (Hamden, CT), and contains all target analytes from the California Department of Cannabis Control's list of regulated solvents at a concentration of 1,000 µg/mL in N,N-dimethylacetamide (DMA). The cannabis sample was purchased from a local dispensary. All dilutions/extractions were done with HPLC-grade DMA (Sigma-Aldrich®, St. Louis, MO).

All standard sample preparation and handling was conducted in a properly ventilated fume hood for safety and to reduce opportunities for solvent contamination from other sources in the laboratory. Additionally, the tapered borosilicate vials were baked out in a GC oven at 150 °C for 10 minutes.

The 1,000 µg/mL standard was transferred to a clean tapered vial and capped with a Mininert® valve. Serial dilutions of 500, 100, 50.0, 10.0, 5.00, 1.00, 0.500, 0.100, and 0.050 µg/mL were done by pipetting an appropriate amount of DMA into a tapered vial, sealing the vial with a Mininert valve, and then using a gas-tight syringe to transfer an appropriate volume of standard sample between vials.

For sample extraction, 0.5 g of cannabis was put into a scintillation vial with 10 mL of DMA and sonicated for 10 minutes. Aliquots for analysis were taken directly from the sample extract. For analysis, a gas-tight syringe was used to transfer 20 µL of either the standard sample or cannabis sample to a 20 mL headspace vial, which was quickly capped after the aliquot was added.

GC Conditions

Column: Zebron™ ZB-624*PLUS*™
Dimension: 30 meter x 0.25 mm x 1.40 µm
Part No.: 7HG-G040-27
Injection: Split (10:1) @ 250 °C
Recommended Liner: Zebron PLUS 4 mm ID single taper with wool
Liner Part No.: AG2-0A11-05
Carrier Gas: Helium @ 1.5 mL/min (Constant Flow)

Oven Program:

Ramp(°C/min)	Temp (°C)	Time(min)
-	30	6.0
15	85	0.0
45	250	2.0

Detector: JEOL JMS-Q1500GC GC-MS

MS Conditions

Ionization Mode: EI^+
Ionization Energy: 70 eV
Ionization Current: 100 µA
IS Temperature: 300 °C
GCIF Temperature: 300 °C
Measurement Mode: SIM
Cycle Time: 200 ms
Analysis Time: 15 min

Headspace Conditions

Instrument: HTA HT2800T
Syringe Volume: 2.5 mL
Oven Temperature: 60°C
Syringe Temperature: 150 °C
Incubation: 5 min
Sample Volume: 0.50 mL
Fill Volume: 0.80 mL
Pull Ups: 2
Sample Speed: 6 mL/min
Injection Speed: 45 mL/min
Pre-injection Dwell: 1 s
Post-injection Dwell: 3 s
Flush Time: 10 min
Shaker On: 0.2 min
Shaker Off: 0.1 min

Results and Discussion

In this application, we used the Zebron™ ZB-624*PLUS*™ GC column, which is shown to meet the many challenges of residual solvent testing of cannabis products under stringent State of California requirements. These include separation of twenty-one residual solvent analytes of highly variable polarity and boiling point at both high and low levels, reproducibility of peak shape and retention time over multiple analyses owing to the high temperature stability of the Zebron ZB-624*PLUS* GC column, which allows high temperature column bakeout to remove cannabinoid and matrix residues, and separation of closely eluting solvent pairs. However, even with the best GC column, the low-level analysis of residual solvents from cannabis products is highly matrix dependent.

The labeled chromatogram for the 10 µg/mL standard sample (**Figure 1**) shows that each component was detected. Most components were completely separated; however, Methanol/Ethylene Oxide, Isopropanol/Acetonitrile, and Benzene/1,2-Dichloroethane are well-known to be difficult to separate on a 624 column. **Figure 2** shows the SIM chromatograms for these compounds showing that even though they are not completely separated on the TICC, SIM is powerful enough to detect and quantitate these compounds. The compound at the top of each pair contains no interfering ions, and the interfering ions in the bottom chromatograms are suppressed enough for suitable separation and quantitation of the target analyte.

Example calibration curves for six compounds are shown in **Figure 3**, and linearity (R^2), RSD, and IDL values are shown in **Table 2**. High linearity ($R^2 > 0.99$) was observed for all compounds within the detectable range except Propane ($R^2 > 0.98$). All area RSDs were less than 10 %, indicating high repeatability and stability. Additionally, the IDL for all compounds was less than 0.2 ppm except Propane and Ethanol, which had IDLs less than 4.5 and 7.5 ppm, respectively. All IDLs were less than the action limits set by the California DCC.

The SIM chromatograms for the Category 1 solvents at 0.5 ppm are shown in **Figure 4**. The quantitative and qualifier ions for all compounds were detected at 0.5 ppm at a signal-to-noise ratio of at least 10:1. Additionally, the IDL for every Category 1 solvent was less than 0.2 ppm, showing that headspace analysis with the JEOL JMS-Q1500GC can meet the requirements for residual solvent testing in California.

The chromatogram for the cannabis sample is shown in **Figure 5**. While it is evident that some residual solvents were detected in the sample, interfering compounds were minimized when compared to analyzing a cannabis sample by liquid injection, due to the nature of headspace analysis. The complexity of the analysis is reduced by limiting sample compounds to only highly volatile analytes.

The quantitative results are listed in **Table 3**. The sample became contaminated by several solvents after sitting in the refrigerator for six months. The SIM chromatograms for Acetonitrile, Dichloromethane, and Trichloroethylene (the three Category 1 solvents detected in the sample) are shown in **Figure 6**. Acetonitrile and Dichloromethane would have both failed the action limits for California testing (indicated in red text in **Table 3**). **Figure 7** shows the three Category 1 compounds compared to the DMA matrix blank. Some native contamination in the matrix blank was observed, but the sample compounds are all more intense than the matrix blank, indicating that the sample was contaminated by other sources. These results provide a good example of how easy it is to contaminate headspace samples, and that this could have been avoided by careful consideration when deciding how and where samples should be stored.

Table 1. List of Target Residual Solvents and Action Limits per the California DCC.

Category 1		Category 2	
Compound Name	Limit (µg/g)	Compound Name	Limit (µg/g)
Benzene	1.0	Acetone	5,000
Chloroform	1.0	Acetonitrile	410
1,2-Dichloroethane	1.0	Butane	5,000
Dichloromethane	1.0	Ethanol	5,000
Ethylene Oxide	1.0	Ethyl Acetate	5,000
Trichloroethylene	1.0	Ethyl Ether	5,000
		Heptane	5,000
		Hexane	290
		Isopropyl Alcohol	5,000
		Methanol	3,000
		Pentane	5,000
		Propane	5,000
		Toluene	890
		Total Xylenes (m- and p-Xylene, and o-Xylene)	2,170

Figure 1. Chromatogram for the 10 ppm Standard Sample Headspace.

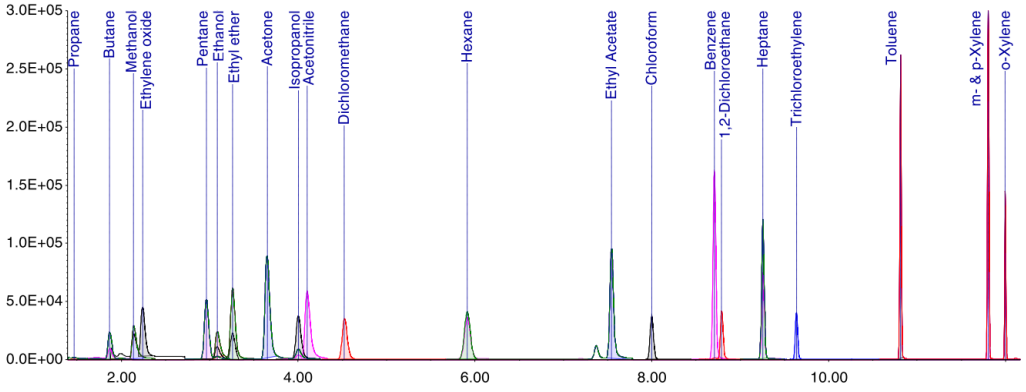


Figure 2. Select SIM Chromatograms for Compounds that are Difficult to Separate. Each Top/Bottom Pair Represent a Set of Peaks Difficult to Separate by Scan Mode Alone.

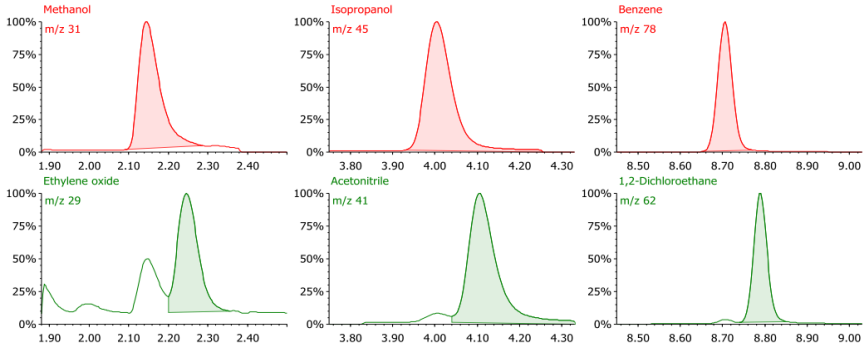


Figure 3. Calibration Curves for Category 1 Solvents.

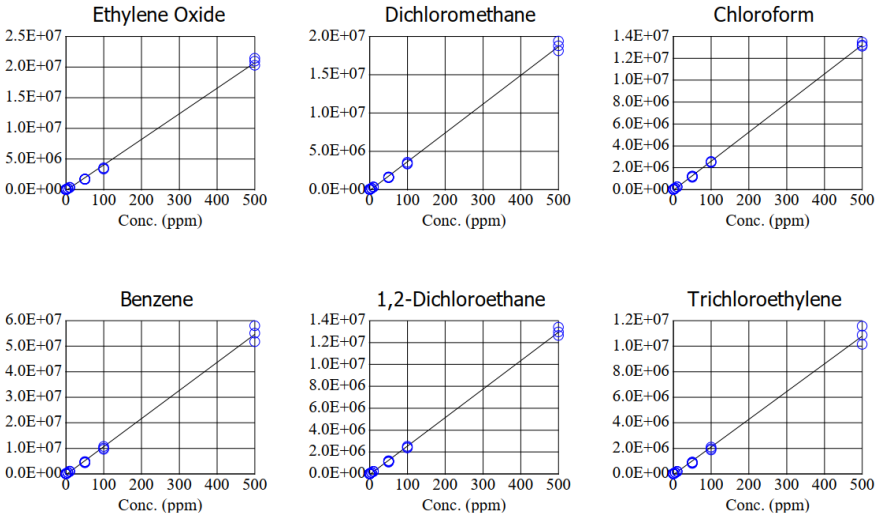


Figure 4. The SIM Chromatograms for Category 1 Solvents at 0.5 ppm Showing Both Quantitative (Red) and Qualifier (Blue) Ions.

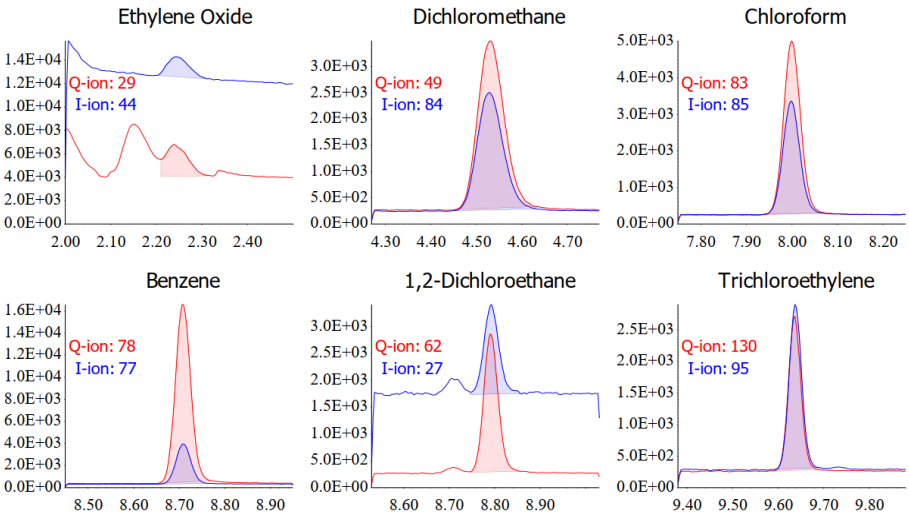


Table 2. Linearity, RSD, and IDL Values for n = 12.

Category 1				Category 2			
Compound Name	R^2	Area RSD	IDL (ppm)	Compound Name	R^2	Area RSD	IDL (ppm)
Benzene	0.9966	5.77	0.08	Acetone	0.9985	1.23	0.02
Chloroform	0.9995	2.89	0.04	Acetonitrile	0.9997	0.72	0.01
1,2-Dichloroethane	0.9989	3.95	0.06	Butane	0.9973	9.54	0.13
Dichloromethane	0.9976	9.02	0.13	Ethanol	0.9980	4.85	7.27
Ethylene Oxide	0.9985	2.39	0.03	Ethyl Acetate	0.9970	1.68	0.02
Trichloroethylene	0.9957	7.69	0.11	Ethyl Ether	0.9970	6.32	0.09
				Heptane	0.9940	3.44	0.05
				Hexane	0.9904	9.69	0.14
				Isopropyl Alcohol	0.9990	6.96	0.10
				Methanol	0.9998	6.36	0.09
				Pentane	0.9848	2.96	4.43
				Propane	0.9919	3.70	0.05
				Toluene	0.9985	7.03	0.10
				m- & p-Xylene	0.9987	8.99	0.13
				o-Xylene	0.9984	8.26	0.12

Figure 5. Chromatogram for the Cannabis Extract Headspace Sample.

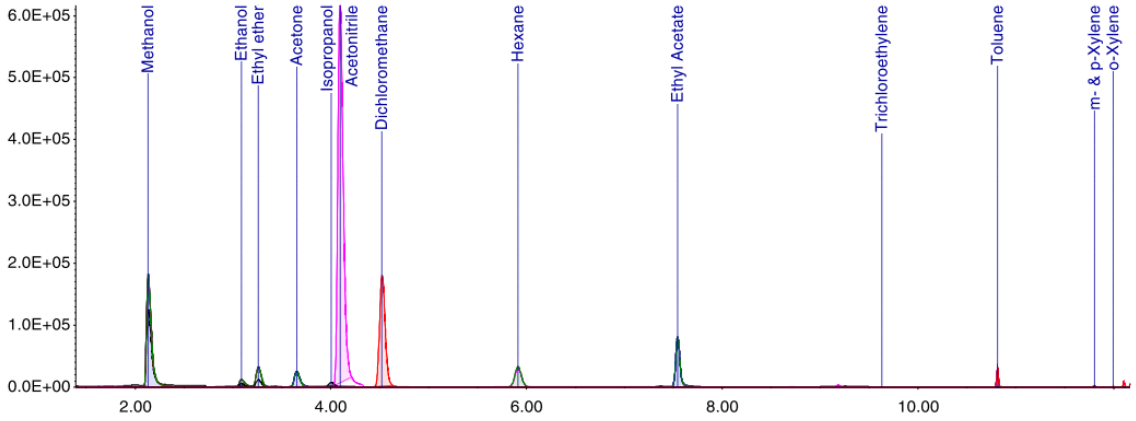


Table 3. Quantitative Results for Analysis of Cannabis Extract.

Compound Name	Concentration (ppm)
Methanol	669
Ethanol	33.3
Ethyl Ether	50.5
Acetone	24.4
Isopropanol	14.1
Acetonitrile	1211
Dichloromethane	488
Hexane	68.1
Ethyl Acetate	79.6
Trichloroethylene	0.22
Toluene	10.0
m- & p-Xylene	0.44
o-Xylene	0.08

Figure 6. The SIM Chromatograms for the Three Category 1 Solvents Detected in the Cannabis Extract with Both Quantitative (Red) and Qualifier (Blue) Ions.

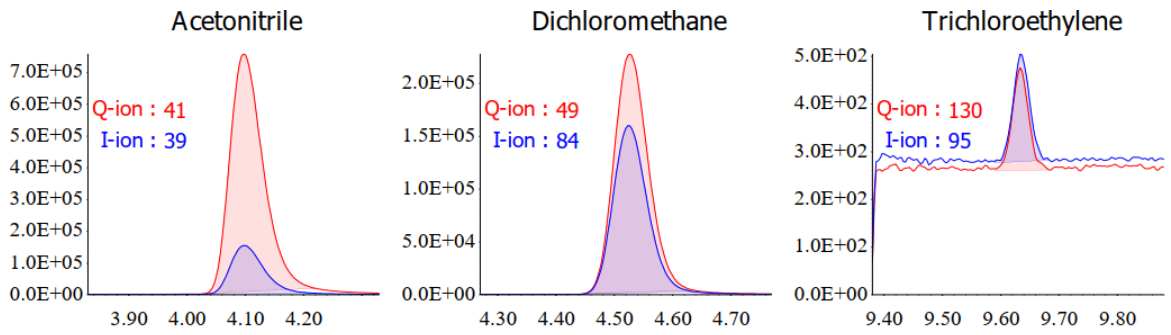
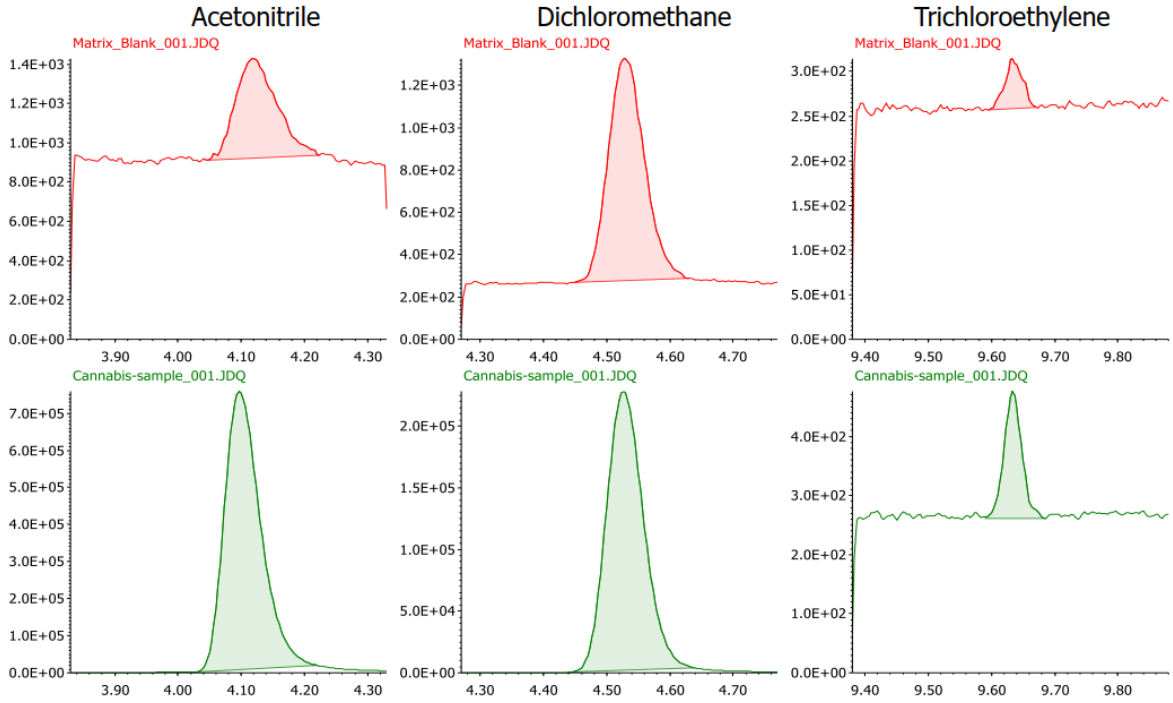


Figure 7. The SIM Chromatograms of the Three Category 1 Solvents Detected in the Cannabis Extract (Bottom, Green) Compared to the DMA Matrix Blank (Top, Red).



Conclusions

Residual solvents were measured in both standard samples and a cannabis sample using headspace analysis. Standard sample results indicated high performance for all analytes with good linearity, area RSD, and IDL. These results also showed that the JEOL JMS-Q1500GC, using a Zebron™ ZB-624*PLUS*™ GC column, can easily handle the Category 1 residual solvents action limits set forth by the California DCC.

Additionally, the cannabis sample, which had been contaminated over time while sitting in storage, would have failed the test for Acetonitrile and Dichloromethane. Good laboratory practices can prevent contamination issues, both in the samples themselves, and during instrumental analysis.

References

1. "Residual Solvents <467>". In: *Chemical Tests*. 30th ed. United States Pharmacopeia. Washington DC: United States Pharmacopeia Convention, 2007.
2. Kolb, Bruno, and Ettre, Leslie S. *Static Headspace-Gas Chromatography: Theory and Practice.* 2nd. Hoboken, NJ: Wiley, May 2006. 384 pp. ISBN 978-0-471-74944-8.
3. Rouseff, Russel L. and Cadwallader, Keith R., eds. *Headspace Analysis of Foods and Flavors: Theory and Practice.* 1st. Advances in Experimental Medicine and Biology. Boston, MA, United States: Springer, 2001. 212 pp. ISBN 978-4615-1247-9. URL: https://doi.org/10.1007/978-1-4615-1247-9.

Zebron™ ZB-624*PLUS*™ GC Columns Ordering Information

ID (mm)	df (µm)	Temp. Limits °C	Part No.
20-Meter			
0.18	1.00	-20 to 300/320	7FD-G040-22
0.25	1.40	-20 to 300/320	7FG-G040-27
30-Meter			
0.25	1.40	-20 to 300/320	7HG-G040-27
0.32	1.80	-20 to 300/320	7HM-G040-31
0.53	3.00	-20 to 300/320	7HK-G040-36
60-Meter			
0.25	1.40	-20 to 300/320	7KG-G040-27
0.32	1.80	-20 to 300/320	7KM-G040-31
0.53	3.00	-20 to 300/320	7KK-G040-36
75-Meter			
0.53	3.00	-20 to 300/320	7LK-G040-36

Zebron PLUS Liners Ordering Information (Compatible with Agilent® GC Systems)

Description	Application	Inlet Style	Dimensions ID x L (mm)	Deactivation	Part No.	Unit
For 5890, 6890 and 7890 Models						
Direct Connect	Trace analysis, Splitless injections	S/SL	4 x 78.5	Standard	AG1-0A50-01 AG1-0A50-05 AG1-0A50-25	ea 5/pk 25/pk
Single Taper	Pesticides	S/SL	4 x 78.5	Standard	-AG1-0A10-01 AG1-0A10-05 AG1-0A10-25	ea 5/pk 25/pk
Single Taper Z-Liner	Semi-volatiles, Dirty samples	S/SL	4 x 78.5	Standard	AG1-0A13-01 AG1-0A13-05 AG1-0A13-25	ea 5/pk 25/pk
Single Taper with Wool	Semi-volatiles	S/SL	4 x 78.5	Standard	AG1-0A11-01 AG1-0A11-05 AG1-0A11-25	ea 5/pk 25/pk
Straight	Volatiles	S/SL	4 x 78.5	Standard	AG1-0A00-01 AG1-0A00-05 AG1-0A00-25	ea 5/pk 25/pk
Straight Z-Liner	Dirty samples, Volatiles, High initial oven temperatures	S/SL	4 x 78.5	Standard	AG1-0A03-01 AG1-0A03-05 AG1-0A03-25	ea 5/pk 25/pk
Straight Single Baffle	Semi-volatiles, Pesticides	PTV	1.8 x 71	Standard	AG1-1F06-01 AG1-1F06-05 AG1-1F06-25	ea 5/pk 25/pk

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