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Analysis of 21 Residual Solvent Residues from Cannabis Matrix by FET Headspace on a Zebron™ ZB-624*PLUS*™ GC Column

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Introduction

While legalization of cannabis-containing products proliferates worldwide, the same products remain on the United States DAN Controlled Substances as Schedule I drugs. As a result, 34 states have legalized the medical and/or recreational use of cannabis products at the time of this publication and are responsible for establishing testing requirements to ensure consumer safety. Twenty-two states have taken the step of enacting mandatory residual solvent testing for all cannabis products, with California's solvent regulations regarded among the most stringent. In California, twenty-one residual solvents, divided into two categories based on their health concerns, are required to be tested in cannabis products. Category 1 compounds have a Minimum Reporting Limit (MRL) of 1 ppm and Category 2 compounds have MRLs ranging from 290 - 5000 ppm.

In the cannabis space, residual solvents are volatile compounds that may have been used in the extraction, processing or refinement of cannabis materials. Analysis of residual solvents in cannabis products presents several significant challenges. The difference in boiling points between the most and least volatile analytes can be over 180 °C. Analytes can range in polarity from nonpolar to highly polar. The variety of cannabis product matrices include several that can trap and retain certain analytes in the sample vials or in the column, necessitating high temperatures to bakeout the compounds prior to analysis. Considering these challenges, a ZB-624*PLUS* GC column was chosen to perform this analysis.

In this technical application note, we demonstrate a method for residual solvent testing that meets California's stringent requirements using the Zebron ZB-624*PLUS* GC column. While 624-type phases are common for pharmaceutical residual solvent testing, cannabis products come with added challenges including very high boiling cannabinoids at percent level concentrations. For this, and other matrix-related reasons, it is beneficial to have the added high temperature stability of the Zebron ZB-624*PLUS* phase to enable a cleaner analysis.

Standard Preparation

200 µL of N, N-Dimethlyacetamide (DMAc) was first added to a 2 mL amber vial fitted with a Mininert valve (Supelco, Bellefonte, PA). 500 µL of California Residual Solvent Mix #2 (Emerald Scientific, San Luis Obispo, CA) was added, making sure to re-seal the Mininert valve upon addition of each component. Next, 1000 µL of California Residual Solvent Mix #1 (Emerald Scientific, San Luis Obispo, CA) was added to the vial and vortexed. This mix is "Standard 1" and is used to prepare subsequent Standards 2, 3, 4, 5 through semi-serial dilution to get the concentration range specified in **Table 1**.

Headspace Autosampler Conditions

Vial: Verex™ Headspace Vial
Description: 23 x 75 mm, 20 mL
Part No.: AR0-3260-13
Vial Seal: 20 mm, PTFE/Silicone, Magnetic (AR0-5255-12)
Instrument: Thermo Scientific® TriPlus™ 500
Vial Incubation Temperature: 170 °C
Vial Incubation Time: 8 min
Loop Temperature: 180 °C
Loop Pressure: 7.5 psi
Loop Equilibration Time: 0.01 min
Injection Mode: Standard
Injection Time: 0.10 min

GC-MS Conditions

Column: Zebron ZB-624*PLUS*
Dimension: 30 meter x 0.25 mm x 1.40 µm
Part No.: 7HG-G040-27
Injection: Split 38:1 @ 250 °C
Split Flow: 75 mL/min
Purge Flow: 5 mL/min
Recommended Liner: Zebron PLUS Liner (Compatible with Agilent® & Thermo Scientific GC instrument), 4 mm ID Single Taper, Wool on Bottom
Liner Part No.: AG2-0A11-05
Carrier Gas: Helium @ 1.3 mL/min (Constant Flow)
Oven Program:

Ramp(°C/min)	Temp (°C)	Time(min)
-	35	2.0
7.3	58	0.0
11.3	115	0.0
25.2	300	8.9

Detector: Thermo Scientific ISQ™ GC-MS/MS
Detector Temperature: 300 °C

Table 1. Analytical Figures of Merit of the Residual Solvent Method on a Zebron™ ZB-624*PLUS*™ GC Column.

Analyte	Retention Time (min)	EI Mass Fragments (m/z)	%RSD Peak Area (n=6)	Calibration Range (µg/mL)	Coefficient of Determination
Propane	1.15	29.2, 38.1, 39.1, 41.1, 42.1	5.79	0.44 to 1192.93	0.998623583
Butane	1.42	29.2, 39.1, 41.1, 42.1, 43.1, 58.1	3.04	1.13 to 2923.08	0.99959537
Methanol	1.62	29.2, 31.1, 32.1	5.86	0.78 to 881.73	0.998637504
Ethylene oxide	1.69	29.2, 42.1, 43.1, 44.1	2.88	0.16 to 355.96	0.999656857
Pentane	2.13	41.1, 42.1, 43.1, 57.1, 72.1	0.81	0.93 to 2576.38	0.99997221
Ethanol	2.26	31.1, 45.1, 46.1	1.43	0.79 to 3471.36	0.999915227
Ethyl ether	2.33	29.2, 31.1, 45.1, 59.1, 74.1	1.36	0.53 to 1738.84	0.999922411
Acetone	2.6	42.1, 43.1, 58	2.81	1.20 to 2621.56	0.999674714
Isopropyl alcohol	2.75	29.2, 43.1, 45.1, 59.1	0.93	1.1 to 1731.67	0.999964032
Acetonitrile	2.86	38.1, 39.1, 40.1, 41.1, 42.1	4.18	1.24 to 209.29	0.999263719
Methylene chloride	3.00	49, 51, 83.9, 86, 88	3.69	0.16 to 444.79	0.999440936
n-Hexane	3.42	41.1, 43.1, 56.1, 57.1, 86.1	3.81	0.06 to 171.38	0.999405304
Ethyl acetate	3.98	43.1, 43.1, 45.1, 61.1, 70.1, 88	1.32	0.43 to 1386.60	0.999927263
Chloroform	4.19	47, 83, 84.9, 86.9, 116.9, 117.9, 118.9	1.21	0.01 to 6.84	0.999938319
Benzene	4.49	74, 76, 77.1, 78.1, 79.1	2.01	0.01 to 3.50	0.999797066
1,2-Dichloroethane	4.54	62, 64, 98, 100	0.78	0.01 to 7.00	0.99996857
Heptane	4.65	41.1, 43.1, 56.1, 57.1, 70.1, 71.14.87	4.19	0.72 to 1706.56	0.999217892
Trichloroethylene	4.87	95, 97, 129.9, 131.9, 133.9	2.51	0.03 to 88.37	0.999741161
Toluene	5.52	39.1, 65.1, 91, 92.1	0.86	0.20 to 520.50	0.999968735
m-xylene and p-xylene	6.14	78.1, 91.1, 103.1, 105.1, 106.1, 107.1	4.06	0.45 to 1025.50	0.999262251
o-xylene	6.27	78.1, 91.1, 103.1, 105.1, 106.1, 107.1	4.02	0.22 to 512.42	0.999281869

Results and Discussion

Table 1 presents the chromatographic figures of merit including retention time to guide identification of individual analytes, EI fragment ions for identification and quantification, and calibration range and precision at working concentration for the developed FET-Headspace GC-MS method on a Zebron ZB-624*PLUS* GC column. The developed method is precise with a 0.7-5.86 % RSD and a linear regression with an R^2 value greater than 0.998 for the 21 solvents.

The separation of 21 residual solvents with symmetric peak and great resolution is shown in **Figure 1**. The robustness of the method was evaluated by analyzing multiple samples followed by standard injection as presented in **Figure 2**. The Zebron ZB-624*PLUS* GC column provided consistent retention, peak shape, and resolution after multiple real sample analyses, proving the robustness of the stationary phase. In addition, low Limit of Detection (LOD) standards (as represented in **Figure 3**) provided sharp peaks and identical retention with low baseline noise. This is possible because of the extensive crosslinking of the stationary phase through ESCTM, that provides steady baseline through low bleed. In addition, the superior deactivated fused silica layer in the column provides inert atmosphere and prevents adsorption of the analyte, thereby providing sharp peaks even at lower levels.

Presented in **Figure 4a-4e** are critical pair separations between residual solvents. **Figure 4a** represents the zoomed chromatogram with 2 critical pairs: Acetone and Isopropyl alcohol, and Acetonitrile and Tetrahydrofuran, with more than baseline resolution. **Figure 4b** shows retention of nonpolar permanent gases propane and butane, indicating the ability of the Zebron ZB-624*PLUS* GC column to retain nonpolar permanent gases in addition to providing selectivity for polar and nonpolar solvents. **Figure 4c** represents another critical pair of Benzene and 1,2-Dichloroethane. This pair requires low level detection as it has a minimum reporting limit of 1 ppm or lower. In addition to chromatographic resolution, spectral transitions help quantify these critical pairs authentically and accurately. Another critical pair that has traditionally been challenging to resolve is Methanol and Ethylene oxide. The presented method utilizes peak focusing with a 35 °C oven temperature, a thick film 624*PLUS* selectivity, and a highly efficient column dimension to bring in complete resolution of this critical pair as shown in **Figure 4d**. A chromatogram demonstrating the balance in selectivity of the Zebrpm ZB-624*PLUS* GC column that provides a balance in resolution for polar and nonpolar compounds is shown in **Figure 4e**.

Figure 1. Separation of Residual Solvents with Clear Resolution and Peak Symmetry on a Zebron™ ZB-624*PLUS*™ GC Column.

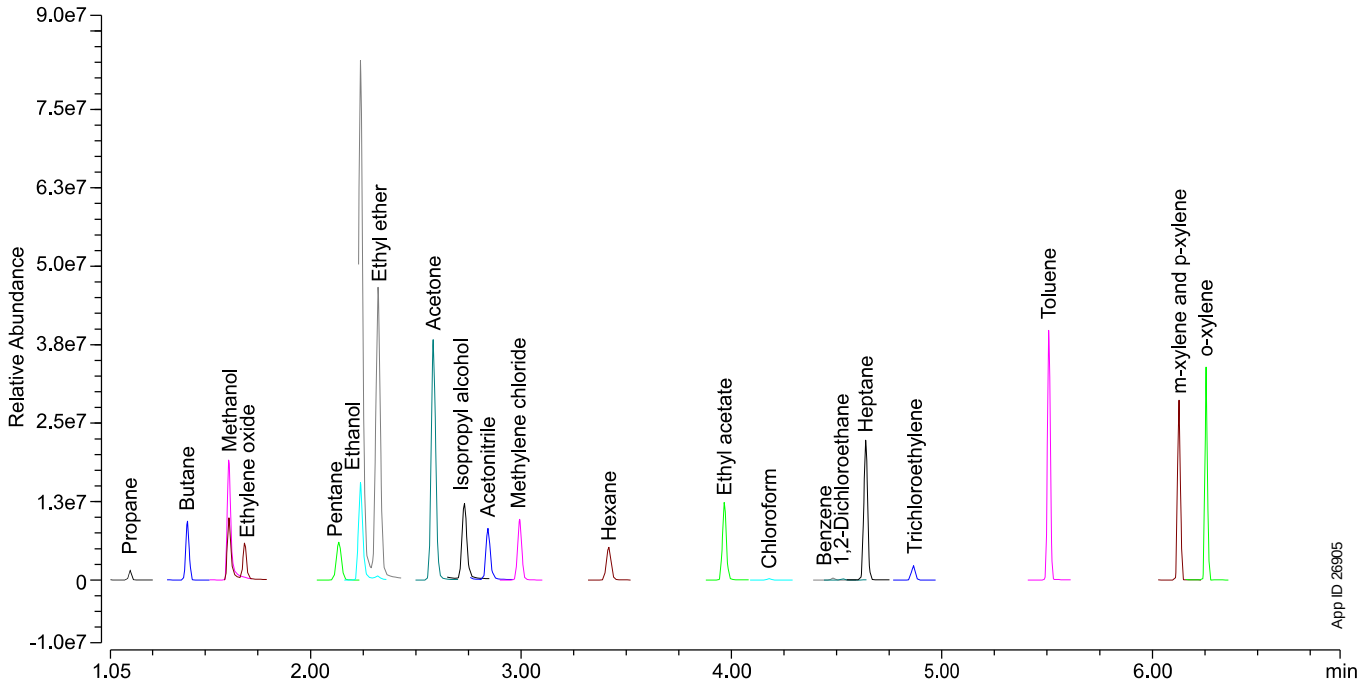


Figure 2. Reproducible Peak Shape and Retention of Residual Solvents Between 11 and 196 Injections of Residual Solvents on a Zebron ZB-624*PLUS* GC Column.

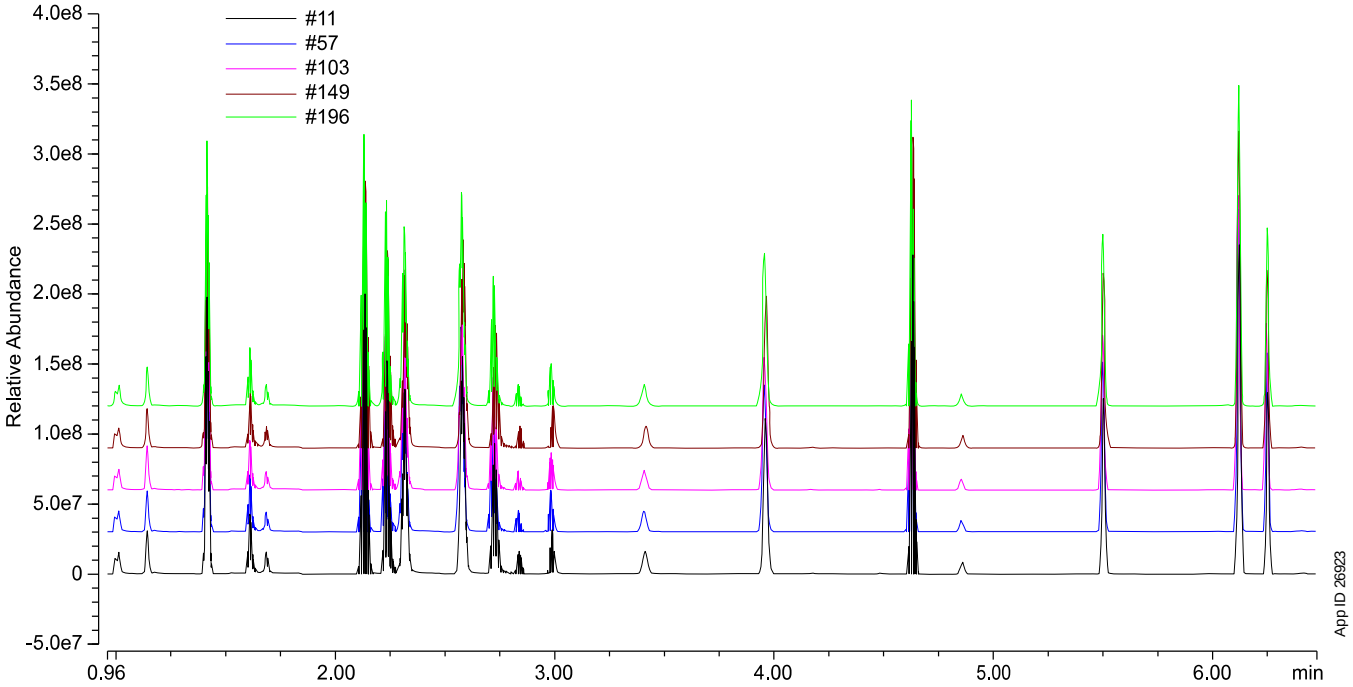


Figure 3. Separation of Residual Solvents at Low LOD on a Zebron™ ZB-624*PLUS*™ GC Column.

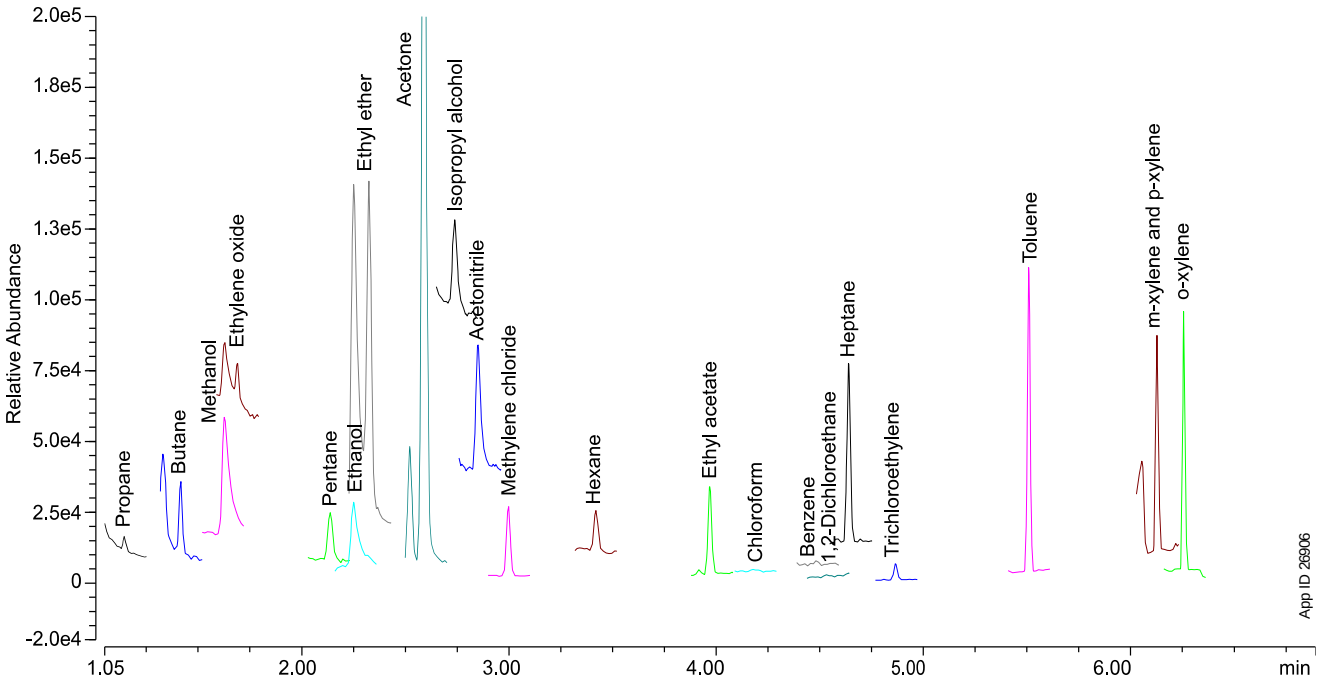


Figure 4a. Chromatogram Showing Resolution of Critical Residual Solvent Pairs on a Zebron ZB-624*PLUS* GC Column that Often Presents Separation Challenges.

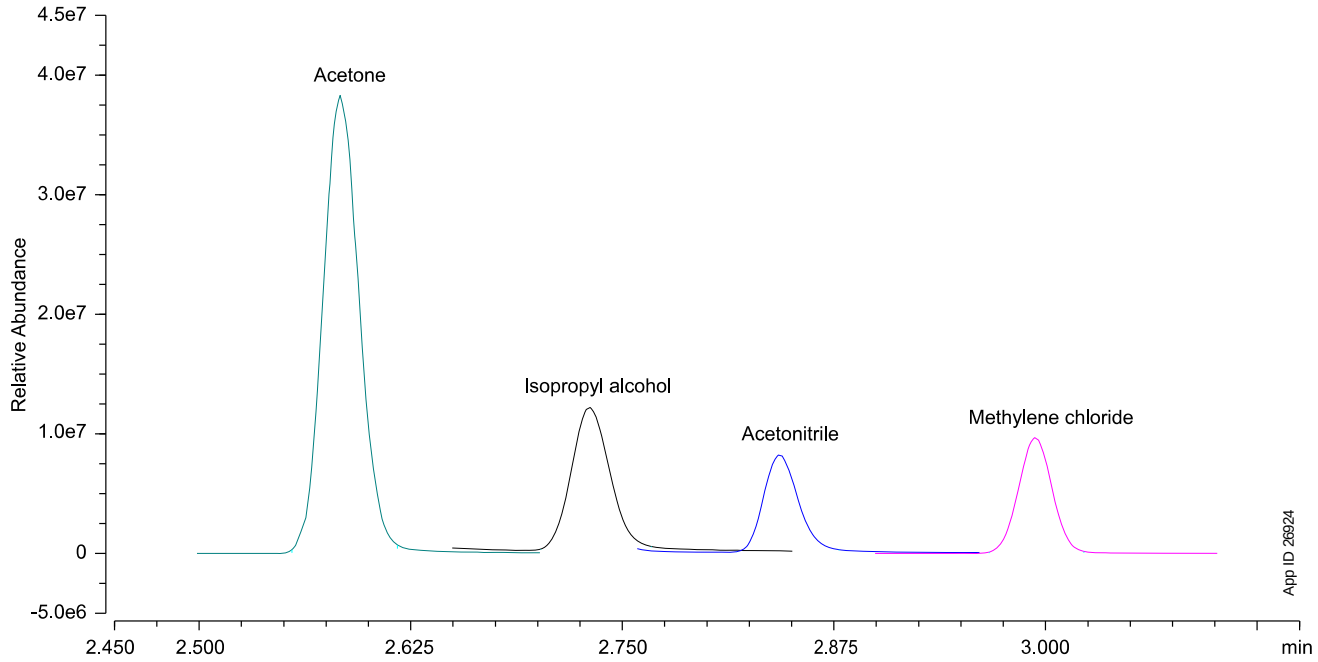


Figure 4b. Chromatogram Showing Resolution of Critical Residual Solvent Pairs on a Zebron™ ZB-624*PLUS*™ GC Column that Often Presents Separation Challenges.

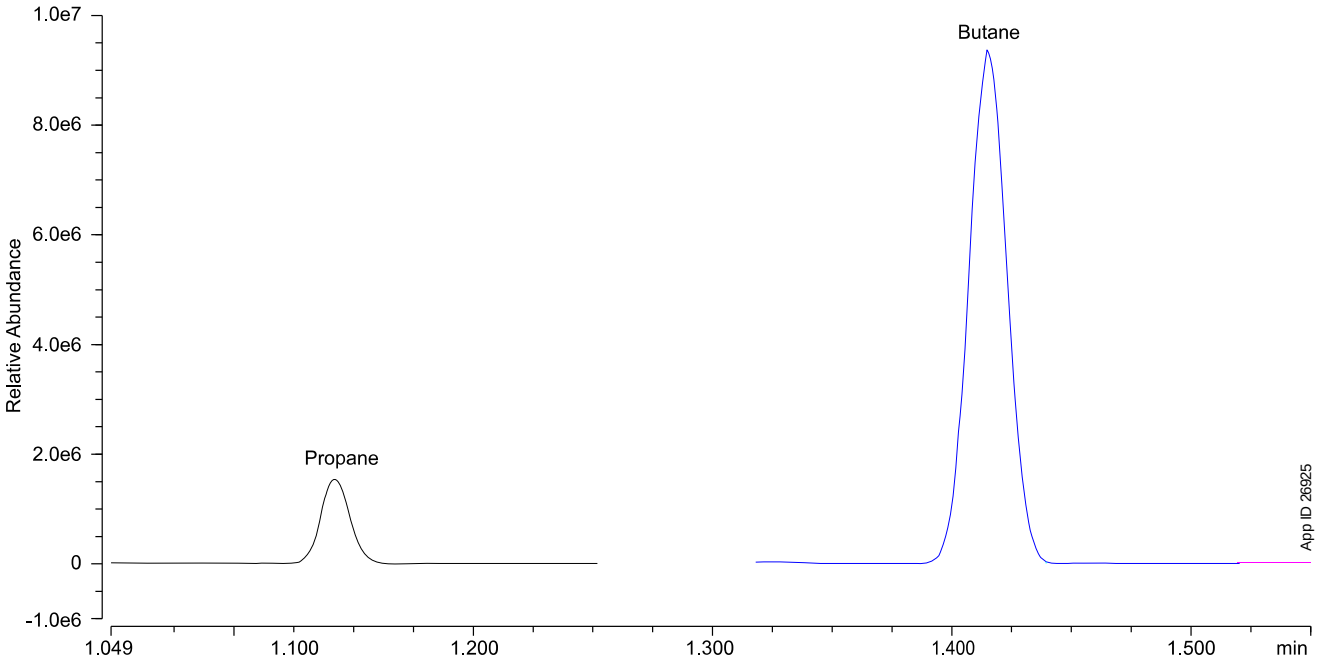


Figure 4c. Chromatogram Showing Resolution of Critical Residual Solvent Pairs on a Zebron ZB-624*PLUS* GC Column that Often Presents Separation Challenges.

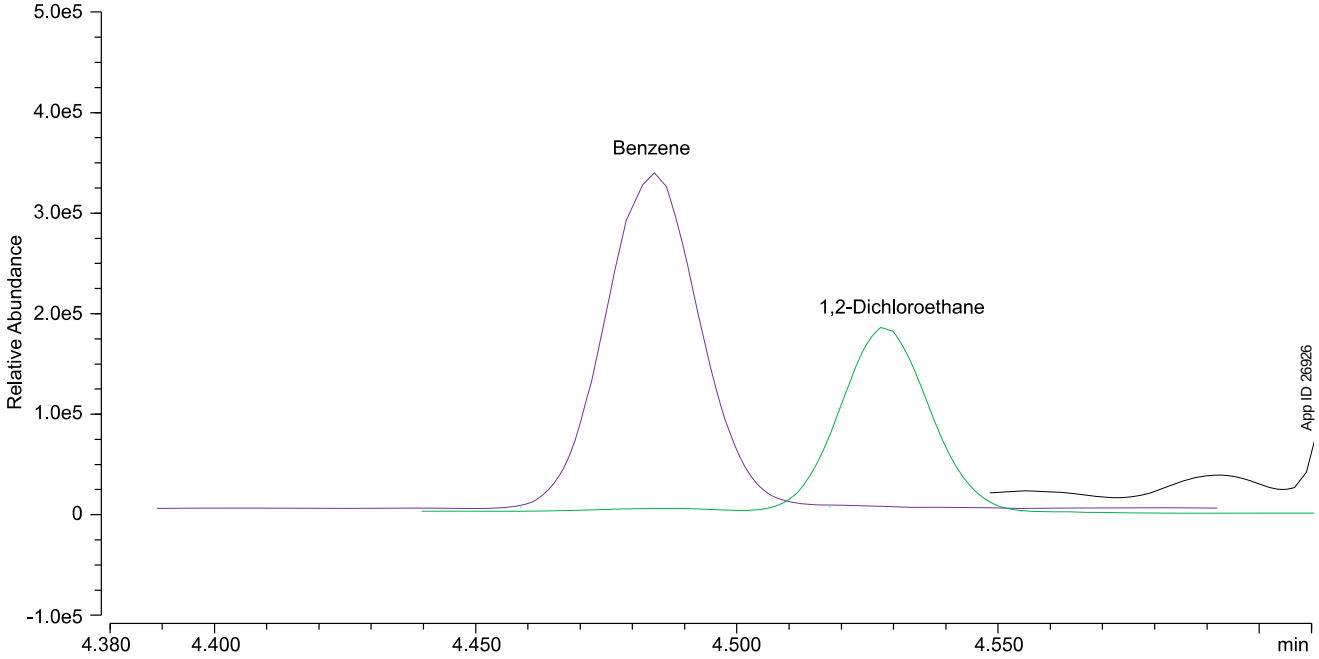


Figure 4d. Chromatogram Showing Resolution of Critical Residual Solvent Pairs on a Zebron™ ZB-624*PLUS*™ GC Column that Often Presents Separation Challenges.

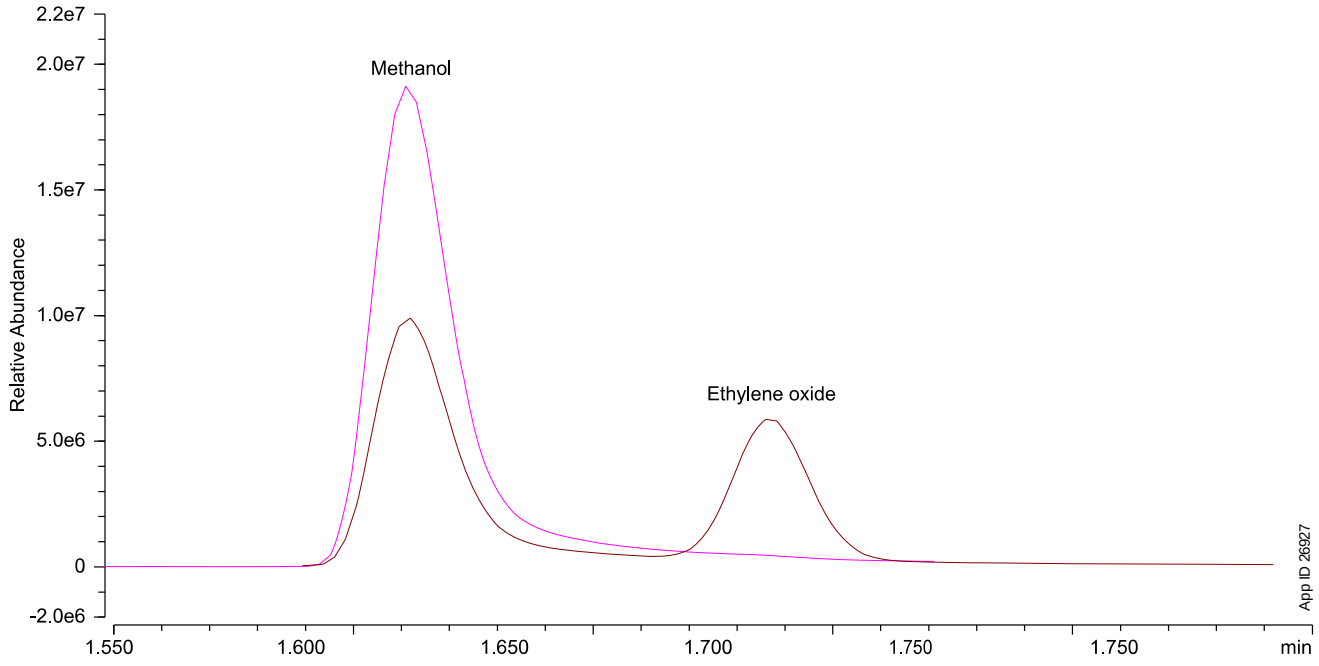
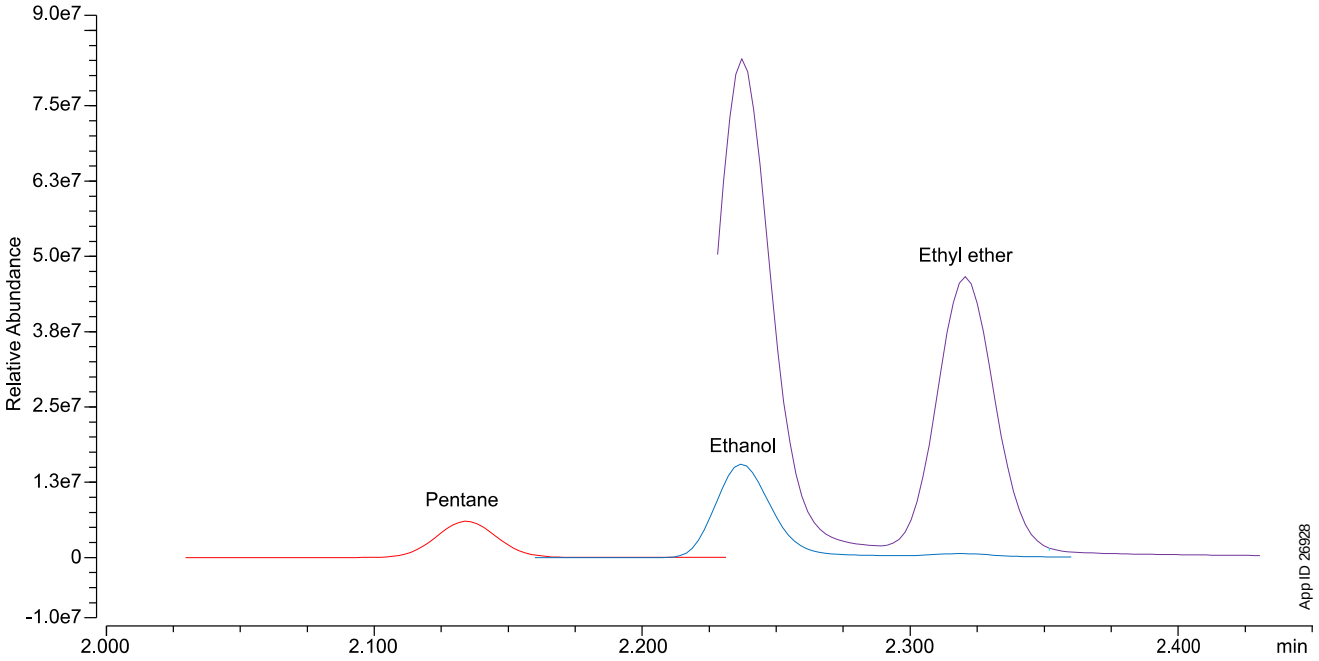


Figure 4e. Chromatogram Showing Resolution of Critical Residual Solvent Pairs on a Zebron ZB-624*PLUS* GC Column that Often Presents Separation Challenges.



Conclusion

The residual solvent method presented in this technical note provides precision, linearity, and resolution of critical pairs. In addition, high temperature resistance of 300/320 °C allows for high temperature bakeout of cannabis matrix components and encourages confident usage of the column on a MS-based detection system. The results here show a reproducible residual solvent analysis on a Zebron™ ZB-624*PLUS*™ GC column. The method provides precision, linearity over a wide range of concentrations, and provides separation of 21 critical residual solvents that are commonly analyzed in cannabis matrices. In addition to resolving critical residual solvent pairs, the Zebron ZB-624*PLUS* GC column provides low bleed, which enables low noise and trace level detection of residual solvents in cannabis.

Ordering Information

Zebron ZB-624*PLUS* GC Columns

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

Note: If you need a 5 in. cage, contact Technical support via Phenomenex.com/chat or simply reach out to your Technical consultant. Conditions may apply. Agilent 6850, some SRI and process GC systems use only 5 in. cages. 0.18 mm, 0.25 mm, and 0.32 mm IDs are MS certified.

Verex™ Headspace Vials

Description	1000/pk
Crimp-Top	
Headspace Vial, 22 x 38 mm, 6 mL Beveled Edge, Flat Bottom, Clear, No Patch	AR0-32F0-13
Headspace Vial, 22 x 38 mm, 6 mL Square Rim, Flat Bottom, Clear, No Patch	AR0-32D0-13
Headspace Vial, 22 x 38 mm, 6 mL Beveled Edge, Round Bottom, Clear, No Patch	AR0-32G0-13
Headspace Vial, 23 x 46 mm, 10 mL Beveled Edge, Flat Bottom, Clear, No Patch	AR0-3220-13
Headspace Vial, 23 x 46 mm, 10 mL Beveled Edge, Round Bottom, Clear, No Patch	AR0-32A0-13
Headspace Vial, 23 x 46 mm, 10 mL Beveled Edge, Round Bottom, Clear, No Patch	AR0-3230-13
Headspace Vial, 23 x 75 mm, 20 mL Beveled Edge, Flat Bottom, Clear, No Patch	AR0-3260-13
Headspace Vial, 23 x 75 mm, 20 mL Beveled Edge, Flat Bottom., Clear, No Patch, Silanized	AR0-3263-13
Headspace Vial, 23 x 75 mm, 20 mL Square Rim, Flat Bottom, Clear, No Patch	AR0-3290-13
Headspace Vial, 23 x 75 mm, 20 mL Beveled Edge, Round Bottom, Clear, No Patch	AR0-3270-13
Screw-Top	
Headspace Vial, 23 x 46 mm, 10 mL 18 mm Screw, Round Bottom, Clear, No Patch	AR0-32H0-13
Headspace Vial, 23 x 46 mm, 10 mL 18 mm Screw, Round Bottom, Amber, No Patch	AR0-32H1-13
Headspace Vial, 23 x 75 mm, 20 mL 18 mm Screw, Round Bottom, Clear, No Patch	AR0-3280-13
Headspace Vial, 23 x 75 mm, 20 mL 18 mm Screw, Round Bottom, Amber, No Patch	AR0-3281-13

Autosampler Compatibility
Flat Bottom: HP / Agilent, Carlo Erba, Shimadzu
Round Bottom: PerkinElmer, Tekmar, LEAP Technologies, Varian

Verex Headspace Crimp-Top Seals / Closures

Description	100/pk
Seal, 20 mm Diameter, PTFE/Gray Butyl Rubber, magnetic cap	AR0-52C5-12
Seal, 20 mm Diameter, PTFE/Gray Butyl Rubber, Pressure Release, silver	AR0-52A0-12
Seal, 20 mm Diameter, PTFE/Butyl Rubber Pharmafix Molded Septum, silver	AR0-52D0-12
Seal, 20 mm Diameter, PTFE/Butyl Rubber Pressure Release, Pharmafix Molded Septum, silver	AR0-52B0-12
Seal, 20 mm Diameter, PTFE/Silicone, magnetic cap	AR0-5255-12
Seal, 20 mm Diameter, PTFE/Silicone, silver	AR1-5250-12
Seal, 20 mm Diameter, PTFE/Silicone Pressure Release, silver	AR0-5220-12

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