Accelerate Inhalants of Abuse Method Development with Pro *EZ*GC Software

By Linx Waclaski

- Model more than 60 common inhalants of abuse—industrial solvents and refrigerants, as well as nitrites and their metabolites—from your desk.
- Develop and optimize methods, achieving instrument-ready conditions within minutes, without taking your instrument offline.
- Use simulated chromatograms to help identify unknown compounds in real-world analyses.

Introduction

Headspace-gas chromatography (HS-GC) is commonly used for the analysis of samples for inhalants of abuse, as well as blood alcohol content, in forensic toxicology laboratories. This analysis typically uses a dual column/dual FID detector setup, with a primary and confirmation column of different selectivities. Since an FID detector does not provide structural elucidation of analytes like a mass spectrometer, method development can be even more time-consuming as individual standards may need to be run to obtain identification of analytes.

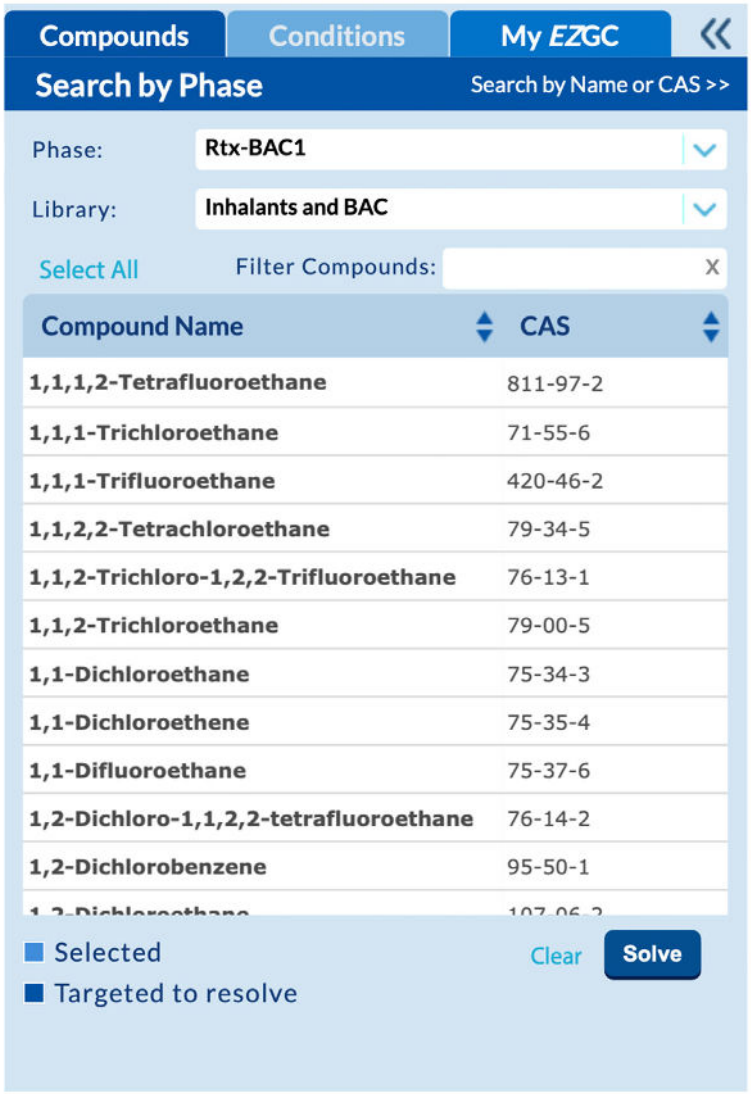
To further compound the difficulty, making changes to method conditions when trying to resolve an entire mix of analytes of varying chemical classes can potentially lead to retention order shifting, requiring re-confirmation using single standards. The Pro *EZ*GC chromatogram modeler can instantly create a method that works for separating your analytes of interest, which can then be entered into your instrument with confidence. Additionally, the modeler's simulated chromatograms can also help you identify unknown peaks in real-world inhalants of abuse analyses. Restek has libraries of 60-plus inhalants of abuse on four unique column phases: Rtx-BAC1, Rtx-BAC2, Rtx-BAC Plus 1, and Rtx-BAC Plus 2.

How to use the Pro *EZ*GC chromatogram modeler

The Pro *EZ*GC chromatogram modeler can be accessed at www.restek.com/pro*ez*gc, where you will be asked to log in or create an account. Creating an account is free and allows simulated models be saved to access later. After logging in, the software's interface will appear. On the left, choose to either "Search by Name or CAS," where a list of compounds you want to separate can be entered into the text box below (Figure 1A), or "Search by Phase," where you can select a Restek column and then choose from a list of compounds modeled on that particular phase (Figure 1B). When selecting the first option, the modeler will return results for all columns that your compounds are modeled on. Then, select the column that provides the best resolution or select whichever column(s) you have on hand.

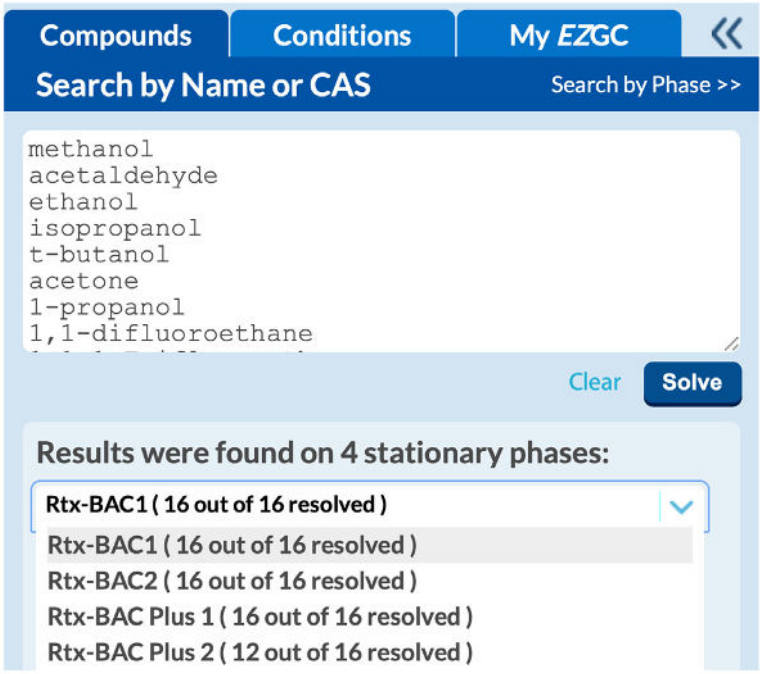
Figure 1A: By default, Pro *EZ*GC will let you search by entering the compounds you want to separate into the text box.

Figure 1B: Alternatively, you can search by a particular column stationary phase.



As an example, what if an analysis needs to separate a standard blood alcohol resolution check mix; several commonly abused refrigerants found in "air dusters": some "poppers" (commonly abused volatile nitrite compounds); and their alcohol metabolites? Begin by entering these compounds and then click the "Solve" button. (Figure 2). The software will return a list of all columns that these analytes are modeled on while stating how many of the compounds are fully resolved on each column.

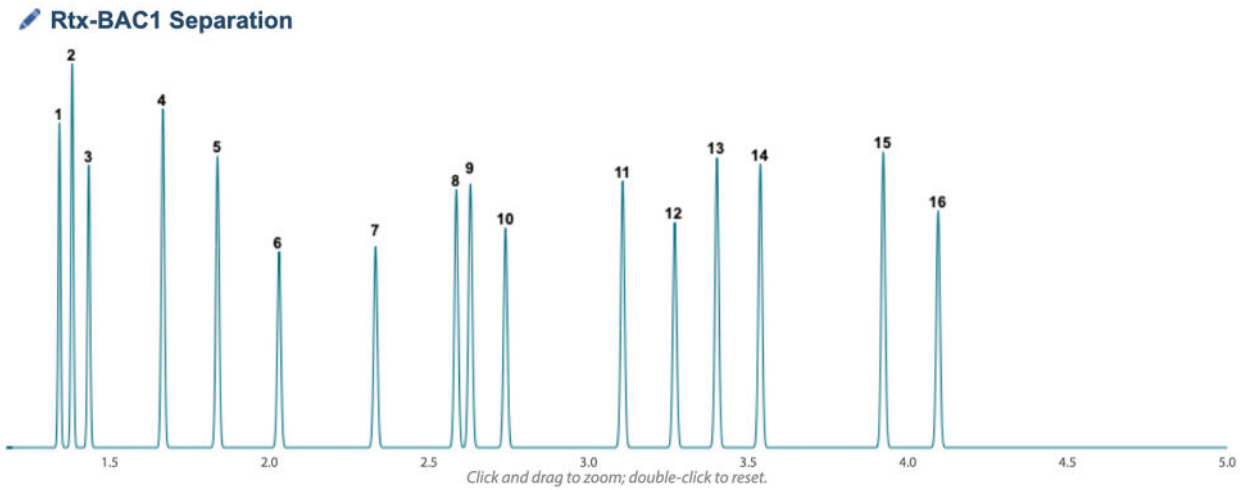
Figure 2: Upon clicking "Solve," a list of columns will appear, each stating how many compounds they were able to resolve.



The compounds in this example shows results on four different columns, with three of them having complete resolution of all 16 compounds. The Rtx-BAC Plus 2 column only resolved 12 out of 16 compounds. Based on this, a dual column set of Rtx-BAC1 and Rtx-BAC2 columns would be recommended since they achieve complete resolution of these particular compounds.

After selecting the desired column from the list, a simulated chromatogram will instantly appear on the right side of the interface showing the separation on the column selected. Below the simulated chromatogram are the suggested method conditions to achieve the separation (Figure 3). The compound table, displayed below the model, provides predicted retention time (t_R), predicted resolution (R_S), peak width, and elution temperature (T_{peak}).

Figure 3: Simulated chromatogram with peaks listed in the compound table below.



Column: **Rtx-BAC1**, 30 m, 0.32 mm ID, 1.8 µm (cat.# 18003)
Carrier Gas: Helium, Constant Flow @ 2.56 mL/min
Average Velocity: 39.70 cm/sec
Outlet Pressure (abs): 14.70 psi
Oven Temp.: 35 °C (hold 1 min) to 165 °C @ 30 °C/min

Peaks	t_R (min)	R_s	Peak Width (min)	T_{peak} (°C)	Peaks	t_R (min)	R_s	Peak Width (min)	T_{peak} (°C)
1. 1,1,1-Trifluoroethane	1.34	2.3	0.017	45.3	9. Acetone	2.63	1.7	0.026	83.9
2. 1,1,1,2-Tetrafluoroethane	1.38	2.3	0.017	46.5	10. 1-Propanol	2.74	4.2	0.026	87.2
3. 1,1-Difluoroethane	1.44	2.9	0.018	48.0	11. Isobutyl nitrite	3.11	6.2	0.026	98.2
4. Methanol	1.67	7.9	0.021	55.0	12. Isobutyl alcohol	3.27	5	0.026	103.1
5. Acetaldehyde	1.84	7.9	0.023	60.1	13. n-Butyl nitrite	3.40	5	0.026	107.1
6. Ethanol	2.03	8.3	0.024	65.9	14. n-Butyl alcohol	3.54	5.2	0.026	111.2
7. Isopropanol	2.33	10	0.025	75.0	15. Isoamyl nitrite	3.92	6.5	0.026	122.7
8. t-Butanol	2.59	1.7	0.026	82.6	16. Isoamyl alcohol	4.09	6.5	0.026	127.8

If you are satisfied with the provided solution, the suggested method parameters can be entered into the instrument software, ready for use. The Pro *EZ*GC modeler has created a separation method for the compounds of interest without the instrument needing to be taken offline.

It's important to note that analyses of compounds such as these will generally use headspace sample introduction. This can have an influence on peak width and resolution compared to a direct injection. Therefore, it's possible some resolution values the software calculates will be less due to peak broadening from headspace introduction. Optimizing headspace parameters is required for best results.

How to Make Custom Changes to a Method in Pro *EZ*GC Software

The Pro *EZ*GC chromatogram modeler will suggest an optimized method of separation by default, but changes can be made to the suggested method with the results updating in real time after each change is made. Examples include switching carrier gas from helium to hydrogen, changing column dimensions, altering carrier gas flow rates, and changing oven temperature ramp rate.

To make custom changes to a method, click on the "Conditions" tab on the left side of the interface. The software solves for "Speed" by default, locking some parameters. To unlock all parameters for customization, click the "Custom" radio button found in the "Results" section. All fields will turn white, allowing all parameters to be changed.

Changing method conditions is demonstrated in Figures 4 and 5 by changing the carrier gas from helium to hydrogen, slightly increasing the flow rate, and increasing the oven temperature ramp rate. As seen on the simulated chromatogram, this reduces the analysis time by over thirty seconds per analysis (compare to Figure 3), while still providing resolution of all compounds.

Figure 4: To make custom changes to a method, click on the "Conditions" tab and then select the "Custom" radio button under "Results," All fields can now be edited.

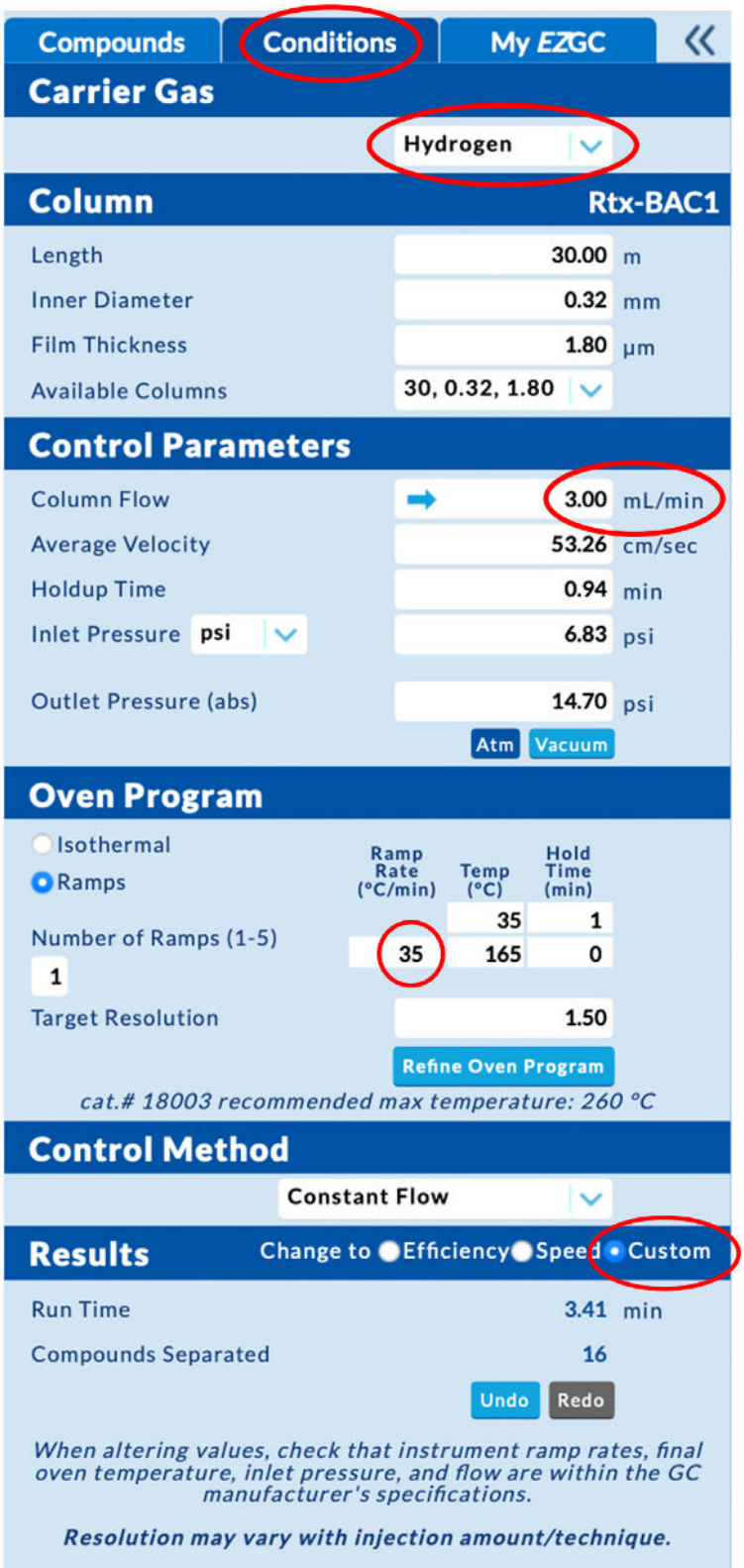
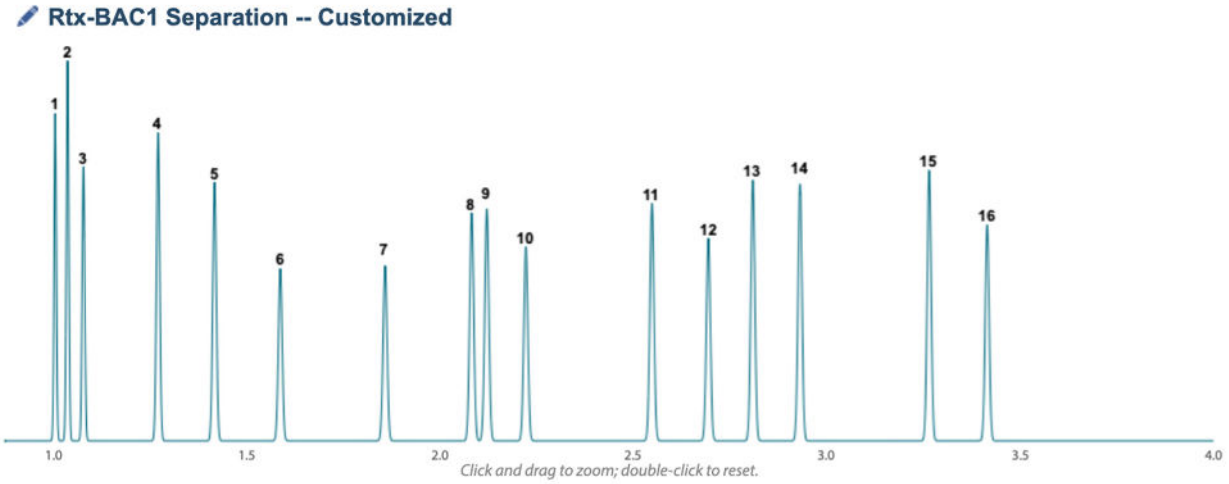


Figure 5: Resultant simulated chromatogram after making manual changes as shown in Figure 4.



Column: **Rtx-BAC1**, 30.00 m, 0.32 mm ID, 1.80 µm (cat.# 18003)
Carrier Gas: Hydrogen, Constant Flow @ 3.00 mL/min
Average Velocity: 53.26 cm/sec
Outlet Pressure (abs): 14.70 psi
Oven Temp.: 35 °C (hold 1 min) to 165 °C @ 35 °C/min

Peaks	t_R (min)	R_s	Peak Width (min)	T_{peak} (°C)	Peaks	t_R (min)	R_s	Peak Width (min)	T_{peak} (°C)
1. 1,1,1-Trifluoroethane	1.00	2.4	0.013	35.2	9. Acetone	2.12	1.6	0.024	74.2
2. 1,1,1,2-Tetrafluoroethane	1.04	2.4	0.014	36.3	10. 1-Propanol	2.22	4.3	0.023	77.8
3. 1,1-Difluoroethane	1.08	2.9	0.015	37.7	11. Isobutyl nitrite	2.55	6.3	0.023	89.2
4. Methanol	1.27	7.7	0.019	44.5	12. Isobutyl alcohol	2.69	5	0.023	94.3
5. Acetaldehyde	1.42	7.7	0.021	49.6	13. n-Butyl nitrite	2.81	5	0.023	98.3
6. Ethanol	1.59	8.2	0.022	55.6	14. n-Butyl alcohol	2.93	5.3	0.023	102.6
7. Isopropanol	1.86	9.7	0.023	65.0	15. Isoamyl nitrite	3.27	6.6	0.023	114.3
8. t-Butanol	2.08	1.6	0.023	72.9	16. Isoamyl alcohol	3.42	6.6	0.022	119.5

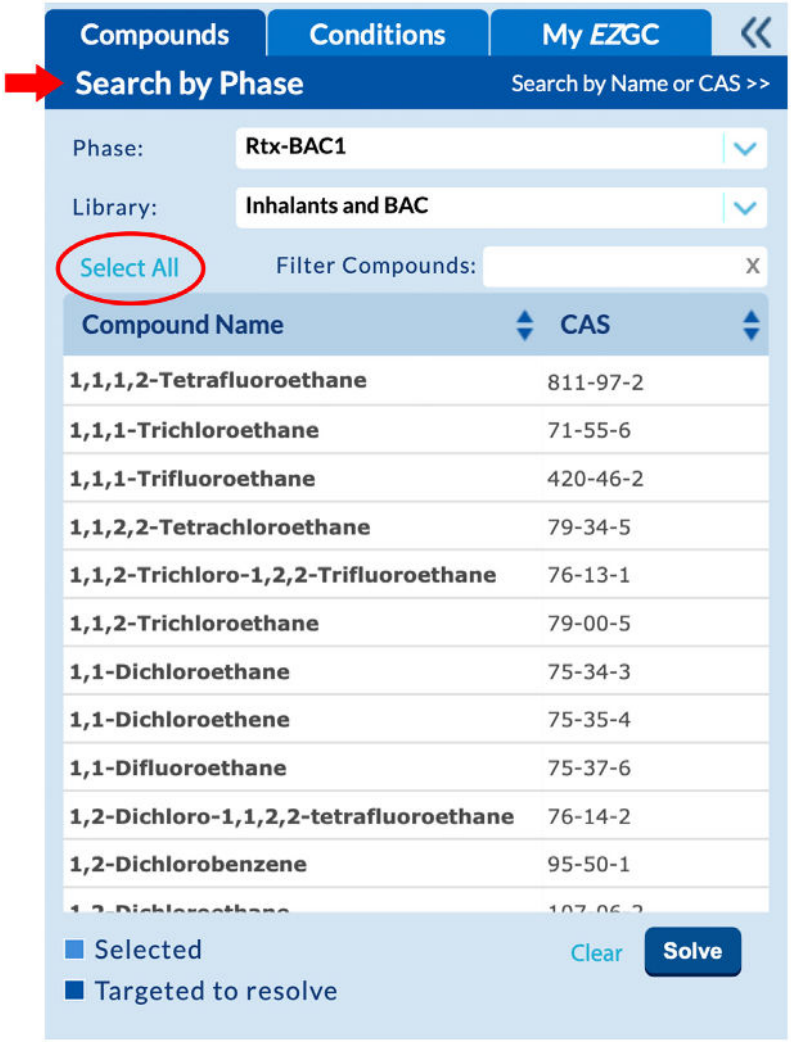
How to Use Pro *EZ*GC software to Help Identify an Unknown Peak

Given the number of inhalants that can potentially be abused, it's unlikely that a toxicology laboratory will calibrate for all possibilities. Therefore, a routine analysis could end up revealing an unknown peak.

In the following example, a blood sample that's analyzed by headspace does not show an expected ethanol peak. The analysis does, however, show an unknown peak with a retention time that does not match what the instrument has been calibrated for. The instrument is set up for a dual column/dual FID method, limiting the ability to gather structural information for the compound, and a GC-MS is not readily available. In this instance, Pro *EZ*GC software may be able to help.

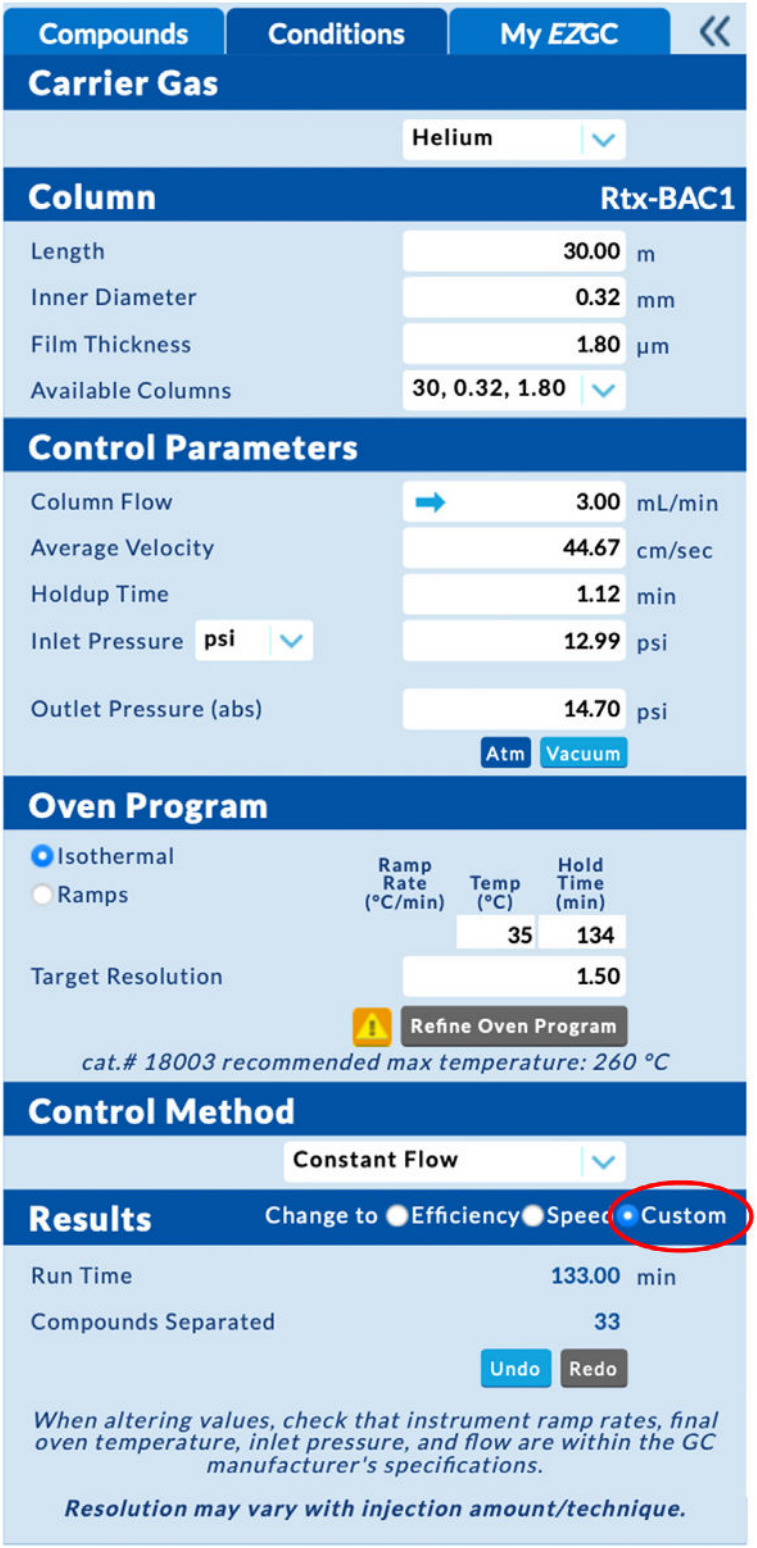
To see if the unknown compound could potentially be modeled in the Pro *EZ*GC modeler, start by logging into the software and then click on "Search by Phase." Use the drop-down list to select the Restek column used in the analysis. In this example, the Rtx-BAC 1 column has been selected. Under the "Library" drop-down menu, select "Inhalants and BAC." After this, click on "Select All," which will select every compound that is modeled on that phase in the chromatogram modeler (Figure 6). Then, click the "Solve" button.

Figure 6: Under the "Search by Phase" section, clicking the "Select All" button is a quick way to select all compounds in the library.



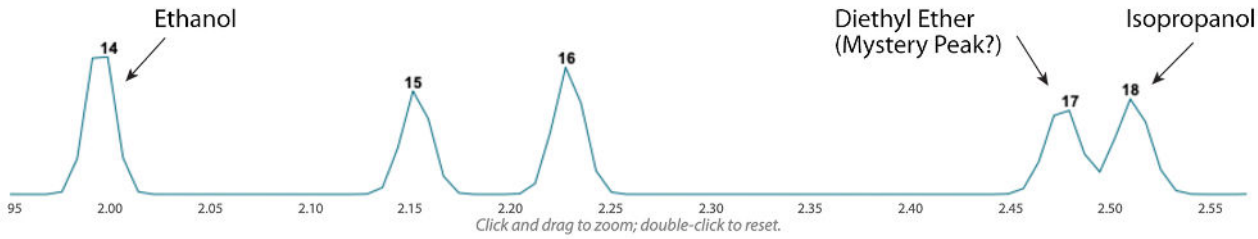
Next, click on the "Conditions" tab at the top. Now, within the "Conditions" tab, click the "Custom" radio button under "Results" at the bottom. The method conditions used on the instrument for the analysis can now be entered. Figure 7 shows an isothermal method at 35 °C, with a helium carrier gas flow of 3 mL/min.

Figure 7: Selecting the "Custom" radio button within the "Conditions" tab will let you enter custom method parameters.



Now that the modeler is set up with the conditions, it can be used to investigate the unknown peak in the blood sample. In this case, the unknown peak eluted after ethanol, but before isopropanol, though very close to it. By Zooming in on this portion of the model chromatogram by clicking and dragging the cursor over the model shows that diethyl ether theoretically elutes immediately before isopropanol (Figure 8). While this is not a positive confirmation by any means, this information can be used to obtain a sample of diethyl ether and analyze it to see if the unknown compound's retention time matches the diethyl ether standard. Note, because this is a dual column method, keep in mind that the retention time would have to match on both columns. If it does, then the unknown peak has been identified.

Figure 8: Zooming into the simulated chromatogram reveals a potential ID of the unknown peak.



Column: **Rtx-BAC1**, 30.00 m, 0.32 mm ID, 1.80 µm (cat.# 18003)
Carrier Gas: Helium, Constant Flow @ 3.00 mL/min
Average Velocity: 44.67 cm/sec
Outlet Pressure (abs): 14.70 psi
Oven Temp.: 35 °C (hold 134 min)

Peaks	t_R (min)	R_s	Peak Width	T_{peak} (°C)
14. Ethanol	2.00	2.7	0.030	35.0
15. Trichloromonofluoromethane	2.15	2.2	0.033	35.0
16. C5	2.23	2.2	0.034	35.0
17. Diethyl ether	2.48	0.9	0.038	35.0
18. Isopropanol	2.51	0.9	0.039	35.0

Conclusion

The Pro *EZ*GC chromatogram modeler is an excellent tool for offline method development. It lets you instantly develop a method of separation without having to touch your instrument, saving time and money. It can also help identify an optimal column or column set for the separation you are trying to achieve. Restek has a large library of inhalants of abuse on the Rtx-BAC1, Rtx-BAC2, Rtx-BAC Plus 1, and Rtx-BAC Plus 2 columns. In addition, if a mass spectrometer is not available, Pro *EZ*GC software can assist in identifying a mystery peak that you haven't calibrated for. Get started with the Pro *EZ*GC chromatogram modeler today at www.restek.com/pro*ez*gc

RESTEK
Pure Chromatography

Questions? Contact us or your local Restek representative (www.restek.com/contact-us).

www.restek.com

Lit. Cat.# CFAR3506-UNV