Blood Alcohol and Inhalants of Abuse Analysis Using Gas Chromatography Optimized with Computer Software.

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Introduction

Screening for volatile inhalants of abuse, as well as analyzing blood alcohol content, is commonly performed in forensic toxicology laboratories using headspace gas chromatography with flame ionization detection (HS-GC-FID). The analyses are generally performed using dual columns with specialized stationary phases that optimally separate these volatile compounds. While separation profiles of standard blood alcohol screening compounds are usually well characterized by column manufacturers on these application specific columns, elution profiles of inhalants may not be as readily available. In addition, providing example chromatograms with static run conditions may not suit laboratories, who want to experiment with faster run times, column dimensions, carrier gases, etc.

These issues can be solved by using computer modeling software to predict retention times of compounds of interest on various stationary phases. In addition to the ability of the web-based software to help select a column and provide an optimized separation of compounds of interest on a specific stationary phase, the software can also be used to make changes to analytical conditions and observe the effect on elution, making it a valuable tool for method development and optimization.

Figure 1: Comparison of instrument confirmation run retention times with computer predicted retention times for Rtx-BAC1 and Rtx-BAC2. There is excellent agreement, with all predicted retention times being < 0.5% different than actual.

Rtx-BAC1 Compound	Instrument Confirmation Run RT	Model Prediction RT	% Difference
1,1,1-trifluoroethane	1.159	1.16	0.086%
1,1,1,2-tetrafluoroethane	1.191	1.193	0.168%
C3	1.222	1.224	0.164%
difluorochloromethane	1.23	1.231	0.081%
1,1-difluoroethane	1.236	1.236	0.000%
dichlorodifluoromethane	1.26	1.26	0.079%
iso-butane	1.351	1.351	0.148%
1,2-dichloro-1,1,2,2-tetrafluoro ethane	1.391	1.391	0.000%
methanol	1.437	1.437	0.070%
C4	1.447	1.447	0.069%
acetaldehyde	1.595	1.595	0.000%
fluorodichloromethane	1.721	1.721	0.058%
ethyl chloride	1.734	1.734	0.000%
ethanol	1.788	1.788	0.112%
trichloromonofluoromethane	1.907	1.907	0.052%
C5	1.958	1.958	0.000%
diethyl ether	2.11	2.11	0.000%
isopropanol	2.133	2.133	0.141%
1,1-dichloroethene	2.204	2.204	0.136%
methylene chloride	2.325	2.325	0.129%
1,1,2-trichloro-1,2,2-trifluoro ethane	2.373	2.373	0.000%
tert-butanol	2.462	2.462	0.122%
acetone	2.521	2.521	0.159%
acetonitrile	2.536	2.536	0.000%
trans-1,2-dichloroethene	2.633	2.637	0.152%
n-propanol	2.689	2.692	0.112%
MTBE	2.838	2.838	0.070%
C6	2.931	2.931	0.102%
1,1-dichloroethane	2.954	2.954	0.034%
chloroform	3.226	3.226	0.155%
isobutyl nitrite	3.277	3.277	0.092%
isobutyl alcohol	3.574	3.574	0.252%
methyl ethyl ketone	3.788	3.788	0.290%
ethyl acetate	3.769	3.769	0.212%
carbon tetrachloride	3.846	3.846	0.078%
1,1,1-trichloroethane	3.853	3.853	0.182%
n-butyl nitrite	3.821	3.821	0.157%
1,2-dichloroethane	3.92	3.92	0.127%
benzene	4.023	4.023	0.124%
1-butanol	4.107	4.107	0.097%
C7	4.348	4.355	0.161%
bromodichloromethane	4.465	4.472	0.157%
trichloroethene	4.492	4.5	0.178%
1,2-dichloropropane	4.795	4.802	0.146%
isoamyl nitrite	4.912	4.918	0.122%
2-pentanone	5.244	5.254	0.191%
2-chloroethyl vinyl ether	5.264	5.273	0.171%
isoamyl alcohol	5.309	5.322	0.245%
toluene	5.776	5.783	0.121%
1,1,2-trichloroethane	5.838	5.851	0.222%
dibromochloromethane	5.885	5.896	0.187%
C8	5.993	6.002	0.150%
methyl isobutyl ketone	6.185	6.196	0.178%
tetrachloroethene	6.339	6.35	0.173%
2-nitropropane	6.339	6.353	0.221%
2-hexanone	6.998	7.011	0.186%
chlorobenzene	7.22	7.235	0.208%
bromoform	7.366	7.384	0.244%
ethylbenzene	7.349	7.362	0.177%
m-xylene	7.517	7.533	0.213%
p-xylene	7.517	7.533	0.213%
C9	7.662	7.676	0.183%
o-xylene	7.972	7.988	0.201%
1,1,2,2-tetrachloroethane	8.14	8.155	0.184%
C10	9.262	9.277	0.162%
1,3-dichlorobenzene	9.875	9.89	0.152%
1,4-dichlorobenzene	9.937	9.956	0.191%
1,2-dichlorobenzene	10.428	10.445	0.163%
C12	12.172	12.187	0.123%
C14	14.719	14.732	0.088%
C15	15.881	15.892	0.069%
C16	16.977	16.988	0.065%
C18	19.055	19.05	0.026%
C20	21.831	21.843	0.055%

Rtx-BAC2 Compound	Instrument Confirmation Run RT	Model Prediction RT	% Difference
1,1,1-trifluoroethane	1.122	1.124	0.178%
C3	1.148	1.148	0.000%
dichlorodifluoromethane	1.16	1.159	0.086%
1,1,1,2-tetrafluoroethane	1.164	1.163	0.086%
1,2-dichloro-1,1,2,2-tetrafluoro ethane	1.193	1.193	0.000%
1,1-difluoroethane	1.193	1.194	0.084%
iso-butane	1.2	1.2	0.000%
difluorochloromethane	1.227	1.226	0.082%
C4	1.25	1.251	0.080%
C5	1.498	1.497	0.067%
trichloromonofluoromethane	1.568	1.569	0.064%
acetaldehyde	1.606	1.606	0.000%
ethyl chloride	1.614	1.613	0.062%
1,1,2-trichloro-1,2,2-trifluoro ethane	1.716	1.722	0.349%
diethyl ether	1.762	1.763	0.057%
fluorodichloromethane	1.83	1.829	0.055%
1,1-dichloroethene	1.917	1.918	0.052%
methanol	1.907	1.908	0.052%
C6	2.034	2.032	0.098%
MTBE	2.322	2.32	0.086%
ethanol	2.423	2.428	0.206%
trans-1,2-dichloroethene	2.474	2.478	0.162%
acetone	2.548	2.548	0.000%
isobutyl nitrite	2.587	2.591	0.154%
methylene chloride	2.61	2.611	0.038%
1,1-dichloroethane	2.969	2.972	0.101%
isopropanol	2.721	2.725	0.147%
tert-butanol	2.873	2.879	0.209%
C7	3.021	3.026	0.165%
n-butyl nitrite	3.13	3.133	0.096%
acetonitrile	3.27	3.273	0.092%
carbon tetrachloride	3.262	3.265	0.092%
1,1,1-trichloroethane	3.384	3.386	0.059%
ethyl acetate	3.406	3.408	0.059%
n-propanol	3.631	3.638	0.193%
methyl ethyl ketone	3.761	3.763	0.053%
chloroform	3.756	3.759	0.080%
Benzene	3.841	3.844	0.078%
isoamyl nitrite	3.984	3.987	0.075%
trichloroethene	4.228	4.232	0.095%
1,2-dichloroethane	4.428	4.429	0.023%
C8	4.386	4.386	0.000%
isobutyl alcohol	4.54	4.549	0.198%
1,2-dichloropropane	4.93	4.935	0.101%
2-pentanone	5.077	5.083	0.118%
n-butyl alcohol	5.164	5.174	0.193%
bromodichloromethane	5.32	5.324	0.075%
toluene	5.464	5.467	0.055%
tetrachloroethene	5.536	5.541	0.090%
2-chloroethyl vinyl ether	5.629	5.633	0.071%
methyl isobutyl ketone	5.817	5.821	0.069%
C9	5.929	5.932	0.051%
isoamyl alcohol	6.268	6.278	0.159%
1,1,2-trichloroethane	6.743	6.748	0.074%
2-hexanone	6.754	6.759	0.074%
ethylbenzene	6.977	6.981	0.057%
dibromochloromethane	7.013	7.019	0.086%
2-nitropropane	7.13	7.136	0.084%
m-xylene	7.103	7.106	0.042%
p-xylene	7.103	7.106	0.042%
chlorobenzene	7.183	7.188	0.070%
C10	7.482	7.487	0.067%
o-xylene	7.653	7.66	0.091%
bromoform	8.717	8.723	0.069%
1,1,2,2-tetrachloroethane	9.589	9.596	0.073%
1,3-dichlorobenzene	9.792	9.796	0.041%
1,4-dichlorobenzene	9.995	10	0.050%
1,2-dichlorobenzene	10.589	10.595	0.057%
C12	10.379	10.382	0.029%
C14	12.941	12.941	0.000%
C15	14.111	14.111	0.000%
C16	15.218	15.216	0.013%
C18	17.26	17.254	0.035%
C20	19.198	19.191	0.036%

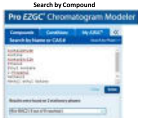
Objectives

- Develop retention time models for inhalants of abuse and blood alcohol analytes of interest on four unique GC stationary phases, using web-based modeling software.
- Verify accuracy of the models against actual analyses.
- Demonstrate the utility of the modeling software for optimizing separations, speeding up methods, and translating to other carrier gases and column dimensions.

Figure 2: The ProEZGC Chromatogram modeler can be accessed at www.restek.com/proezgc. Modeling can be done by entering in compounds of interest or searching by stationary phase and selecting compounds of interest that are modeled on that phase.

BAC Phases Available

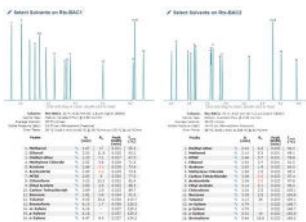
Search by Compound



Search by Phase

Figure 3: By default, the modeler will develop a speed optimized separation on the column you select. Conditions will be presented so that the analyst can replicate the chromatogram. In addition, resolution for each compound is calculated. Below are some examples of various compound classes that are modeled on the Rtx-BAC1 and Rtx-BAC2. a) Select solvents; b) "Poppers" and their metabolites"; c) BAC resolution compounds with internal standards

a) Select Solvents modeled on Rtx-BAC1 (cat.# 18003) and Rtx-BAC2 (cat.# 18002) using ProEZGC Chromatogram Modeler. Optimized for speed.



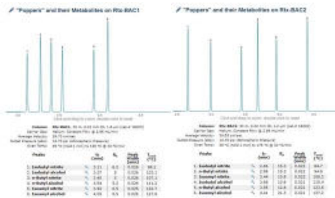
Methods

To build a database for computer modeling of chromatographic separations, the following fused silica capillary columns were installed into an Agilent 7890A GC with a 5975C MSD: Rtx-BAC1, Rtx-BAC2, Rtx-BAC Plus 1 and the Rtx-BAC Plus 2.

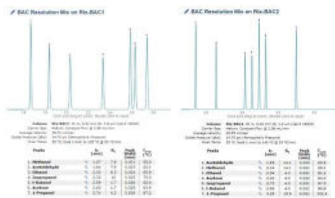
More than 70 volatile inhalants of abuse, including solvents, refrigerants, nitrites (aka "poppers") and their metabolites were analyzed on each column using three different temperature programmed run conditions. Two of the analyses were used to create a retention model based on thermodynamic indices of analytes and the third analysis was used to verify accuracy against the theoretical model **(Figure 1).**

Once the models were finalized, *Pro EZGC,* a web-based modeler, was used to optimize separations on each column, decrease analysis times, translate to different column dimensions or carrier gases, and make user inputted adjustments to parameters such as carrier gas flow rate and oven ramp rates. (**Figure 2, 3, 4**).

b) "Poppers" and their metabolites modeled on Rtx-BAC1 (cat.# 18003) and Rtx-BAC2 (cat.# 18002) using ProEZGC Chromatogram Modeler. Optimized for speed.



c) BAC Resolution Compounds and Internal Standards Rtx-BAC1 (cat.# 18003) and Rtx-BAC2 (cat.# 18002) using ProEZGC Chromatogram Modeler. Optimized for speed.



Results

- Confirmation runs were in excellent agreement with the theoretical modeled analysis, demonstrating acceptable accuracy of the retention time models (**Figure 1**). All compounds showed less than a 0.5% difference for actual vs modeled retention times.
- Selection of various compounds of interest in the software successfully generated optimized separations on each column, allowing the user to choose the column or column set that best fits their needs (**Figure 2** and **Figure 3**).
- Successful demonstration of method optimization using the web-based modeler is provided, including speeding up analyses, translating to a different carrier gas, and user manipulation of parameters (**Figure 4**).

Discussion and Conclusions

Computer modeling of compound retention times in gas chromatography is a valuable tool to aid in column stationary phase selection and method development/optimization. Use of this software greatly reduces the time required for manual method development, as users can input changes to the method and see the results instantaneously. With libraries of 70+ volatile compounds on this dual column set, users can select or input compounds of interest and then calculate elution profiles on each column. The software will present the number of coelutions on each column, allowing the user to select the most appropriate column for their analysis. Libraries are also expandable, and compounds can be added in the future if there is user demand. The solutions presented by the modeler will work on any GC; modeling results are produced on Restek columns.

Additional Data

- Libraries of the same compounds are available on the Rtx-BAC Plus 1 and Rtx-BAC Plus 2 columns. This gives users additional options for selecting the best column for their work, optimizing speed and minimizing coelutions.
- Libraries are live on Restek's website for the Rtx-BAC1, Rtx-BAC2, Rtx-BAC Plus1 and Rtx-BAC Plus2 columns.
- If there is substantial user demand for additional compounds, these can be added to the database.
- Collection of drugs of abuse, including derivatized forms may be collected on other appropriate columns, to further aid and support the forensic toxicology community.

Figure 4: Methods can be customized using the software. In this example the "Poppers" method in Figure 3b was altered to use Hydrogen as a carrier gas on a wider bore, 30 m x 0.53 mm x 3.0 µm df Rtx-BAC1 (cat.# 18001), column. Column flow was manually increased to 6 mL/min to take advantage of the efficiency of hydrogen as a carrier gas. Any of the fields can be altered and users can view the resultant chromatogram to see what the separation looks like. Changes made to the original method (**Figure 3b**) are circled in red.