RESTEK Pure Chromatography

Evaluation of Chromatographic Modelling Software to Streamline GC and LC Method Development

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Introduction

As instrument-based chromatography continues to advance, laboratories are tasked with running larger assays of compounds which presents a number of issues for analysts. One of the largest problems with developing methods suitable for these workflows is finding the instrument and analyst time to perform iterative method development via trialling conditions across a range of variables including stationary phase, mobile phase conditions, temperatures, and flow rates, all of which must combine to give suitable chromatographic resolution.

One approach to minimising these time constraints is the development of computer-based chromatography modelling solutions; which allow panels of analytes to be assessed across a range of conditions without the expense of analyst or instrument time and consumables.

Restek have developed the Pro *EZ*GC and Pro *EZ*LC chromatographic modelers to allow analysts to select from a library of compounds and scout through multiple sets of method conditions so they can predict retention times and ensure sufficient resolution can be achieved for their analytes. In this work we take developed methods from each of the *EZ* suites, and look at how accurately these can be transferred onto laboratory instruments in real world conditions, to test whether there they are viable for real world use.

How Accurate is Pro *EZ*GC?

Pro *EZ*GC was used to model 96 volatile organic compounds in a single assay, covering a range of polarities and chemistries including short chain alcohols and aromatic molecules. The modeller compares a range common stationary phases to select the most appropriate for the panel, offering up alternative choices whilst highlighting any limitations in total number of expected resolved compounds between each phase.

The modelled method conditions were then transferred to an Agilent GC system connected to a Tekmar purge and trap, and utilising an Agilent MSD so that modelled data could be compared with instrument results. In order to account for the additional backpressure caused by the purge and trap, modelled data is shown at a lower constant flow, with instrument flow set according to the expected RT of dichlorodifluoromethane. 2-Picoline is listed in the real data set, though this is not within the model library so comparative data for this compound is not provided.

Acceptance criteria between model and experimental data is set to ±15 seconds, and both datasets are shown below

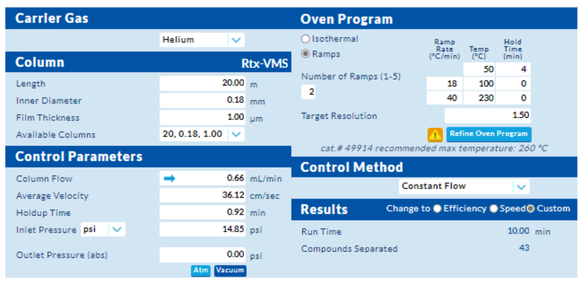


Figure 1 (above) Method conditions as programmed in Pro *EZ*GC User Interface. Table 1 (right): Method conditions developed in *EZ*GC as they were transferred to instrument

Analytical Column:	Rtx®-VMS 20m, 0.18mm, 1.00µm (cat.# 49914)		
Concentrator	Tekmar LSC-3100 Purge and Trap		
Trap	Vocarb 3000 (Type K)		
Purge	11 min. @ 40ml/min (ambient temp)		
Dry Purge	1 min. @ 40ml/min		
Desorb Preheat	245°c		
Desorb	250°c for 2 min. flow 40ml/min		
Bake	260°c for 8 min.		
Interface	0.53mm ID Silcosteel® tubing transfer line 1:40 split at injection port. 1mm ID liner		
Carrier Gas	Helium @ ~1ml/min. in constant flow mode (note – this is set against expected RT for dichlorofluoromethane to account for additional backpressure of purge and trap)		
Detector	Agilent 5973 MSD		
Range	35-300 amu		
Oven Programme:	Temperature (°c)	Ramp (°c/min)	Hold (min.)
	50	0	4
	100	18	0
	230	40	3

Peak #	Compound	Experimental RT (min)	Modeller RT (min)	Difference (s)
1	Dichlorodifluoromethane	1.03	1.03	0
2	Chloromethane	1.13	1.13	0
3	Vinyl Chloride	1.16	1.17	0.6
4	Bromomethane	1.31	1.33	1.2
5	Chloroethane	1.36	1.17	11.4
6	Trichlorofluoromethane	1.43	1.42	0.6
7	Ethanol	1.63	1.75	7.2
8	1,1-Dichloroethene	1.68	1.72	2.4
9	Carbon Disulfide	1.71	1.74	1.8
10	Allyl Chloride	1.93	2	4.2
11	Methylene Chloride	1.99	2.07	4.8
12	Acetone	2.02	2.13	6.6
13	Trans-1,2-Dichloroethene	2.09	2.18	5.4
14	Methyl-Tert-Butyl-Ether	2.15	2.27	7.2
15	Tert-Butyl Alcohol	2.21	2.34	7.8
16	Diisopropyl Ether	2.41	2.54	7.8
17	1,1-Dichloroethane	2.50	2.63	7.8
18	Acrylonitrile	2.53	2.68	9
19	Vinyl Acetate	2.68	2.83	9
20	Allyl Alcohol	2.68	2.85	10.2
21	Ethyl-Tert-Butyl Ether	2.68	2.83	9
22	Cis-1,2-Dichloroethane	2.92	3.08	9.6
23	2,2-Dichloropropane	3.01	3.19	10.8
24	Bromochloromethane	3.09	3.28	11.4
25	Chloroform	3.16	3.37	12.6
26	Ethyl Acetate	3.30	3.51	12.6
27	Carbon Tetrachloride	3.30	3.5	12
28	Methyl Acrylate	3.31	3.52	12.6
29	Propargyl Alcohol	3.34	3.72	22.8
30	Dibromofluoromethane	3.34	3.55	12.6
31	Tetrahydrofuran	3.35	3.54	11.4
32	1,1,1-Trichloroethane	3.36	3.58	13.2
33	2-Butanone	3.50	3.72	13.2
34	1,1-Dichloropropene	3.50	3.71	12.6
35	Benzene	3.79	4.02	13.8
36	Pentafluorobenzene	3.92	4.14	13.2
37	Tert-Amyl-Methyl Ether	3.96	4.2	14.4
38	1,2-Dichloroethane	4.03	4.27	14.4
39	Isobutyl Alcohol	4.14	4.37	13.8
40	Isopropyl Acetate	4.41	4.63	13.2
41	Trichloroethene	4.51	4.73	13.2
42	1,4-Difluorobenzene	4.57	4.78	12.6
43	Dibromomethane	4.97	5.19	13.2
44	1,2-Dichloropropane	5.09	5.19	6
45	Bromodichloromethane	5.17	5.39	13.2
46	Methyl Methacrylate	5.40	5.6	12
47	n-Propyl Acetate	5.56	5.75	11.4
48	2-Chloroethanol	5.75	5.91	9.6
49	Cis-1,3-Dichloropropene	5.84	6.04	12

Peak #	Compound	Experimental RT (min)	Modeller RT (min)	Difference (s)
50	Toluene-d8	6.03	6.22	11.4
51	Toluene	6.08	6.27	11.4
52	Pyridine	6.36	6.5	8.4
53	Tetrachloroethene	6.44	6.63	11.4
54	4-Methyl-2-Pentanone	6.48	6.66	10.8
55	Trans-1,3-Dichloropropene	6.49	6.69	12
56	1,1,2-Trichloroethane	6.63	6.83	12
57	Ethyl Methacrylate	6.69	6.87	10.8
58	Dibromochloromethane	6.79	6.98	11.4
59	1,3-Dichloropropane	6.88	7.06	10.8
60	1,2-Dibromoethane	6.98	7.15	10.2
61	n-Butyl Acetate	7.17	7.32	9
62	2-Hexanone	7.22	7.37	9
63	2-Picoline	7.26	Not modelled	N/A
64	Chlorobenzene-D5	7.39	7.54	9
65	Chlorobenzene	7.4	7.55	9
66	Ethylbenzene	7.44	7.59	9
67	1,1,1,2-Tetrachloroethane	7.46	7.6	8.4
68	m-Xylene	7.55	7.69	8.4
69	p-Xylene	7.55	7.69	8.4
70	o-Xylene	7.82	7.69	7.8
71	Styrene	7.86	7.99	7.8
72	Bromoform	7.86	8	8.4
73	Isopropylbenzene	8.02	8.15	7.8
74	4-Bromo-1-Fluorobenzene	8.18	8.3	7.2
75	Bromobenzene	8.23	8.35	7.2
76	n-Propylbenzene	8.26	8.38	7.2
77	1,1,2,2-Tetrachloroethane	8.3	8.42	7.2
78	2-Chlorotoluene	8.34	8.46	7.2
79	1,3,5-Trimethylbenzene	8.37	8.49	7.2
80	1,2,3-Trichloropropane	8.37	8.49	7.2
81	4-Chlorotoluene	8.43	8.55	7.2
82	Tert-Butylbenzene	8.53	8.65	7.2
83	Pentachloroethane	8.55	8.66	6.6
84	1,2,4-Trimethylbenzene	8.58	8.69	6.6
85	Sec-Butylbenzene	8.63	8.74	6.6
86	p-Isopropyltoluene	8.7	8.82	7.2
87	1,3-Dichlorobenzene	8.73	8.85	7.2
88	1,4-Dichlorobenzene-d4	8.77	8.88	6.6
89	1,4-Dichlorobenzene	8.78	8.89	6.6
90	n-Butylbenzene	8.91	9.02	6.6
91	1,2-Dichlorobenzene	8.99	9.1	6.6
92	1,2-Dibromo-3-Chloropropane	9.38	9.47	5.4
93	Bitrobenzene	9.65	9.74	5.4
94	Hexachlorobutadiene	9.68	9.77	5.4
95	1,2,4-Trichlorobenzene	9.7	9.78	4.8
96	Naphthalene	9.85	9.93	4.8
97	1,2,3-Trichlorobenzene	9.94	10.01	4.2
Average Difference 8.9 seconds, with peak #1 excluded as this was used for RT setting				

Table 2: Data comparison of Pro *EZ*GC modeler vs. empirical data for volatile organic compounds
Note 2-picoline unavailable in Pro EZGC library so no modelled data available

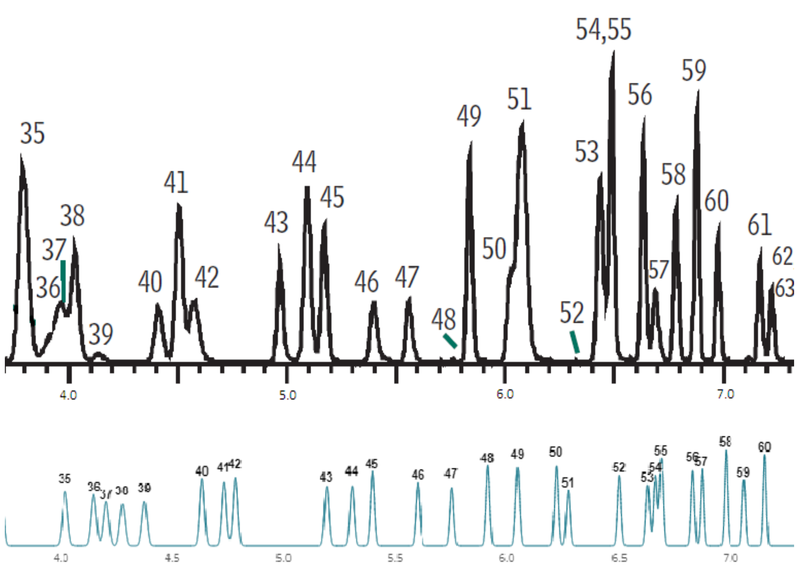


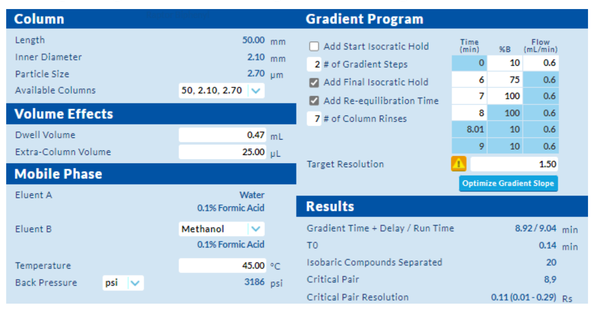
Figure 2: Comparison between acquired (top) data and data as modelled by *EZ*GC (bottom) scaled to approximately same timescale

How accurate is Pro *EZ*LC?

Pro *EZ*LC was used to model a panel of 67 drugs of abuse compounds which are commonly analysed by forensic and toxicology laboratories. These types of panels often contain isobaric compounds which must be separated chromatographically to allow proper quantification. This panel was tested and optimised for use with a 50 x 2.1mm Raptor Biphenyl column, with a target resolution of 1.5 assigned for separation of isobars.

Following the optimization of this model, the method was then transferred to a Shimadzu Nexera LC coupled to a Sciex 4500 LC-MS/MS system to verify the accuracy of RT modelling.

To study the accuracy of the model, an acceptance criterion based on retention time was set to ±15 seconds. This was selected to reflect half the width of a standard MRM (multiple reaction monitoring) retention window; which equates to the time an instrument is acquiring data for ion fragments of target molecules.



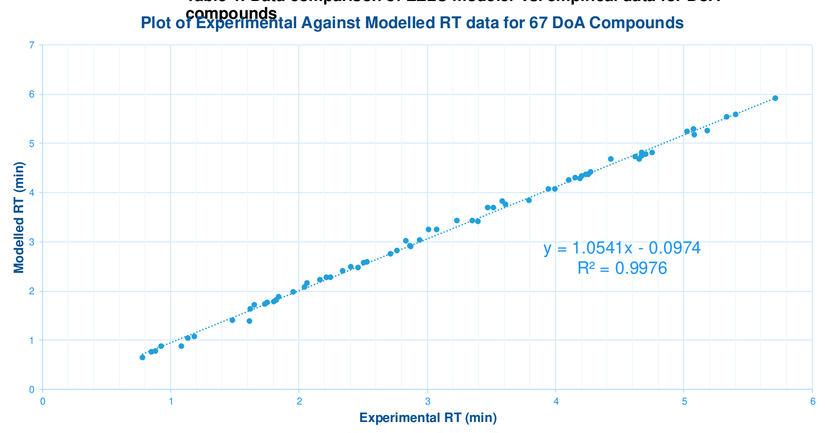
Analytical Column:	Raptor Biphenyl 50 mm x 2.1 mm ID 2.7 µm (cat.# 9309A52)	
Mobile Phase A:	Water, 0.1% formic acid	
Mobile Phase B:	Methanol, 0.1% formic acid	
Column Temp:	45 °C	
Injection Vol:	5 µL	
Diluent:	80:10 MPA(water, 0.1% formic acid): MeOH	
Flow Rate:	0.6 mL/min	
Mode:	Scheduled MRM, ESI (+/-)	
Conditions:	Time(min.)	% B
	0.00	10
	6.00	75
	7.00	100
	8.00	100
	8.01	10
	9.00	10

Figure 3 (above): Method conditions as programmed in Pro *EZ*LC User Interface. Table 3 (right): Method conditions developed in Pro *EZ*LC as they were transferred to instrument.

Peak #	Compound	Experimental RT (min)	Modeller RT (min)	Difference (s)
1	Cotinine	0.78	0.64	8.4
2	Morphine	0.85	0.76	5.4
3	Pregabalin	0.88	0.78	6.0
4	Oxymorphone	0.92	0.87	3.0
5	Hydromorphone	1.08	0.88	12.0
6	Amphetamine	1.13	1.04	5.4
7	Gabapentin	1.18	1.08	6.0
8	Methamphetamine	1.48	1.40	4.8
9	Phentermine	1.61	1.39	13.2
10	Noroxycodone	1.62	1.63	0.6
11	Naloxone	1.65	1.71	3.6
12	Norhydrocodone	1.73	1.73	0.0
13	O-Desmethylcistramadol	1.75	1.76	0.6
14	Codeine	1.80	1.78	1.2
15	MDMA	1.82	1.82	0.0
16	6-Monoacetylmorphine	1.84	1.88	2.4
17	Oxycodone	1.95	1.98	1.8
18	Naltrexone	2.04	2.08	2.4
19	Hydrocodone	2.06	2.16	6.0
20	O-Desmethyl-Venlafaxine	2.16	2.22	3.6
21	6-β-Naltrexol	2.21	2.27	3.6
22	Lamotrigine	2.24	2.28	2.4
23	Ritalinic Acid	2.34	2.40	3.6
24	N-Desmethyltapentadol	2.40	2.49	5.4
25	Norketamine	2.46	2.48	1.2
26	Hydroxybupropion	2.50	2.57	4.2
27	Norfentanyl	2.53	2.59	3.6
28	7-Hydroxyquetiapine	2.71	2.75	2.4
29	Tramadol	2.76	2.82	3.6
30	Benzoylecgonine	2.83	3.02	11.4
31	Zolpidem Phenyl-4-Carboxylic Acid	2.86	2.92	3.6
32	Xylazine	2.87	2.90	1.8
33	Normeperidine	2.94	3.03	5.4

Peak #	Compound	Experimental RT (min)	Modeller RT (min)	Difference (s)
34	Meprobamate	3.01	3.24	13.8
35	7-Aminoclonzepam	3.07	3.25	10.8
36	Phenobarbital	3.23	3.43	12.0
37	Venlafaxine	3.35	3.42	4.2
38	Mirtazapine	3.39	3.41	1.2
39	Butalbital	3.47	3.69	13.2
40	Norbuprenorphine	3.51	3.69	10.8
41	Topiramate	3.58	3.82	14.4
42	LSD	3.61	3.76	9.0
43	AcetylFentanyl	3.79	3.84	3.0
44	Citalopram	3.94	4.07	7.8
45	Desmethyldoxepin	3.99	4.07	4.8
46	Trazodone	4.10	4.25	9.0
47	Haloperidol	4.15	4.30	9.0
48	Dextromethorphan	4.19	4.29	6.0
49	Fentanyl	4.20	4.33	7.8
50	Norfluoxetine	4.23	4.37	8.4
51	PCP	4.25	4.36	6.6
52	Buprenorphine	4.27	4.42	9.0
53	Carisoprodol	4.43	4.67	14.4
54	Paroxetine	4.62	4.73	6.6
55	Duloxetine	4.65	4.68	1.8
56	EDDP	4.67	4.74	4.2
57	Cyclobenzaprine	4.67	4.75	4.8
58	Nortriptyline	4.67	4.81	8.4
59	Sufentanil	4.70	4.78	4.8
60	Amitriptyline	4.75	4.81	3.6
61	Lorazepam	5.02	5.24	13.2
62	Oxazepam	5.07	5.28	12.6
63	Methadone	5.08	5.17	5.4
64	Dehydroaripiprazole	5.18	5.25	4.2
65	Alpha-OH-Alprazolam	5.33	5.53	12.0
66	Nordiazepam	5.40	5.59	11.4
67	Temazepam	5.71	5.91	12.0
Average difference 6.3 seconds				

Table 4: Data comparison of *EZ*LC modeler vs. empirical data for DoA compounds



Conclusions

Comparing the dataset from Pro *EZ*GC against instrumentally acquired data, there was a strong positive correlation across the entire 96 compound set, with an R^2 value of 0.9992. Almost all compounds met the passing criteria (99%) and on average the retention time error between modelled and real-world data was 8.9 seconds. Data from Dichlorodifluoromethane was excluded from this comparison, as the compound was used to account for the required difference in flow rates due to the inclusion of the purge and trap. The average calculated difference places the majority data points within the typical RT windows used for GC-MS methodology, meaning transfer of method from model to instrument is relatively straightforward. However, care should be taken when transferring compounds, as it is possible to obtain lower than expected results due to inaccurate RT windows, as opposed to a true loss of detection sensitivity.

Propargyl alcohol was the only compound from the dataset which fell outside of the predicted RT windows by greater than 15 seconds, with an RT difference of 22.8 seconds. Whilst this is an outlier in the current dataset, it does highlight the need for careful consideration when transferring methods from model to instrument, as there is the potential for loss of individual analytes which could lead to incorrect results being reported in the final method.

In the comparison of the Pro *EZ*LC dataset and data acquired following method transfer to an instrument, all 67 compounds were accurately modelled within the ±15 seconds criterion, with a 6.3 second difference on average between the two data sets. This demonstrates accuracy within the Pro *EZ*LC model, and also ease of transfer to an LC-MS/MS system, with even the extremities of the data falling within the typical MRM window allowing smooth transfer from model to testing. One caveat to note regarding transfer from prediction to experimental is that close attention should be paid the parameters for dwell volume and extra column volume, as these impact how accurately RT can be calculated, and are instrument dependent parameters.

Within this panel there are a total of 10 sets of isobars, with *EZ*LC tasked with achieving a resolution of 1.5 for these sets. Pro *EZ*LC produced a dataset in which, 8 groups of isobars could be separated as prescribed; and a further set indicated a resolution of 0.9, indicating slight overlap, though these could be separated via different quantifying ions. Phentermine and methamphetamine however presented a resolution of 0.11, indicating peaks which were not resolved. Within the real data set, these compounds were resolved much better, with a difference in RT of 7.8 seconds between these isobars. This difference in resolution is proposed to be due the process used for compiling the library of compounds, and improvements are scope for future works.

As demonstrated, both Pro *EZ*GC and Pro *EZ*LC can accurately predict and model RT for a large number of analytes within assays. This allows further transfer to real world testing within laboratories whilst minimising instrument and analyst downtime. The accuracy of RT prediction shown by both modellers demonstrates the ease of transfer for these methods, highlighting how beneficial these software can be across a range of industry sectors, including but not limited to environmental and forensic toxicology laboratories.

Future considerations include expanding libraries and consideration of additional stationary phases, though any further feedback for suggested improvements is always welcomed.

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