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Tutorial for the Excel spreadsheet for Lab Exercise 1 Isocratic chromatography

You must have a basic understanding of the Hydrophobic Subtraction model (HS) and the Linear Solvent Strength model (LSS) in order to use this spreadsheet.

There are 4 functions (but you do not *need* to use 1) and 3)):

1) Predict the retention times for the HS experiment (Sheet: Predict tR).
2) Run the experiments for HS and calculate the results (Sheet: HS Calculations).
3) Predict the retention times for the LSS experiments (Sheet: HS Calculations).
4) Run the experiments for LSS and calculate the results (Sheet: LSS Calculations).

1) Predict retention times for HS experiments (Predict tR)

There are two uses of this function:

a) The experiments must be run under standard conditions according to the lab manual, but you might change the flow-rate, if the time for the experiment is too long or short. So you can predict the expected min and max retention time.
b) To help in assigning the compounds to the peaks, the order of retention can be predicted, even if the predicted retention times are not accurate.

To use this function:

1) Copy-Paste the column parameters for your column from the sheet: Table values (or enter the numbers by hand) to cell A7:L7. The parameter C7.0 is not used by the spreadsheet.
2) Enter the run conditions in cell O5:07. You may not know the exact number of theoretic plates (N) and the dead time (tM), but use a qualified guess or just N = 5000 and tM = 1. Tmax is just for the X-axis in the plot.

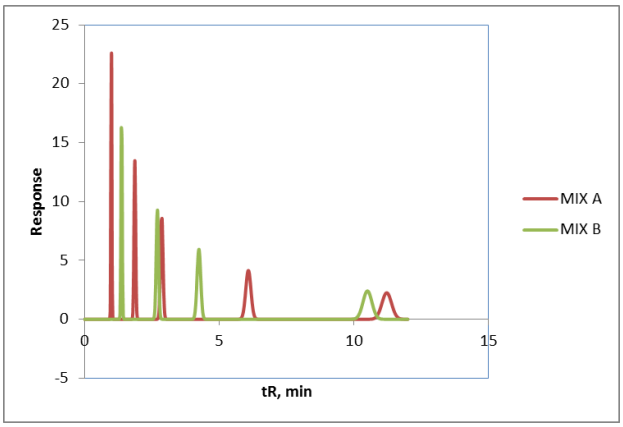
Example: For the column 414 Clipeus C18:

id	name	manufact	nufacture	type	H	S*	A	B	C2.8	C7.0	retention
414	Clipeus C1	Higgins Ar	25	B	1	0	-0.04	-0.01	0.07	0.34	9.5

Run conditions:	
N	4000
tM	1
tmax	12

The result is:

	Calculated results HS-conditions 50 % ACN Clipeus C18	tR	w
MIX A	Thiourea	1.00	0.06
	Amitriptyline	1.87	0.12
	n-butylbenzoic acid	6.08	0.38
	Benzonitrile	2.87	0.18
	trans-Chalcone	11.22	0.71
MIX B	NN-Diethylacetamic	1.38	0.09
	Ethylbenzene	10.50	0.66
	Acetophenone	2.71	0.17
	Anisole	4.25	0.27



2) Run the experiments for HS and calculate the results (HS Calculations):

There are two uses of this function:

a) Determination of the HS-parameters for the column.
b) Calculation of F_s for the column compared to table values or other columns.

Run the experiments and enter the results:

1) Enter the run conditions in cell D4:L7.
2) Enter the experimental retention times in cell D11:D19.

Example a):

Enter:		Run conditions:
	Column	Clipeus C1 Higgins Analytical
	Eluent	MeCN:H2= 50:50
	Temp	35 oC
	Comments	

		Enter experimental values here tR
MIX A	Thiourea	0.67
	Amitriptyline	1.25
	n-Butylbenzoic acid	4.12
	Benzonitrile	1.92
	trans-Chalcone	7.51
MIX B	NN-Diethylacetamide	0.92
	Ethylbenzene	6.80
	Acetophenone	1.81
	Anisole	2.85

The results for the column are (cell F28:K31):

HS Parameters for the column:					
H	S*	A	B	C(2.8)	k(EB)
0.986	0.014	-0.060	0.007	0.055	9.15

Example b):

Copy-Paste Value or enter table values or HS-parameters for other columns in cell A38:L43 (one line for each column) and see the results (column comparison parameter Fs):

Comparison of columns - Fs												
id	name	manufacturer	nufacture	type	H	S	A	B	C28	C70	retention	Fs
0	Clipeus C18				0.986	0.014	-0.060	0.007	0.055	NA	9.15	
414	Clipeus C18	Higgins Ar	25	B	1	0	-0.04	-0.01	0.07	0.34	9.5	**3.12**
415	Clipeus C8	Higgins Ar	25	B	0.82	-0.01	-0.18	0.02	0.09	0.24	4.9	**5.96**
416	Clipeus Cyano	Higgins Ar	25	CN	0.42	-0.06	-0.42	0.01	0.05	0.78	1.2	**14.91**
417	Clipeus Phenyl	Higgins Ar	25	phenyl	0.58	-0.11	-0.3	0.03	0.07	0.48	2.5	**15.64**

The second line is a comparison of the experimental values to the table values for the same column (should be 0, if the column were 'perfect')

3) Predict the retention times for the LSS experiments (HS Calculations)

For LSS a number of isocratic runs must be done at different eluent strengths, and this may take a long time for low values of the strong eluent.

To predict the run time before performing the experiment you may look at the lower part of this sheet from row 55 and downwards:

1) A table of predicted retention times for all elution strengths, $0 \leq \phi \leq 1$, is given in D74:N83.
2) To see the predicted chromatogram, enter a % B in cell G57.
3) The spreadsheet uses the experimental retention time for Thiourea as the dead time tM. If you have a better value for tM you can override the experimental value by entering the new value in C58. Just delete the contents in C58 to restore to the experimental value.

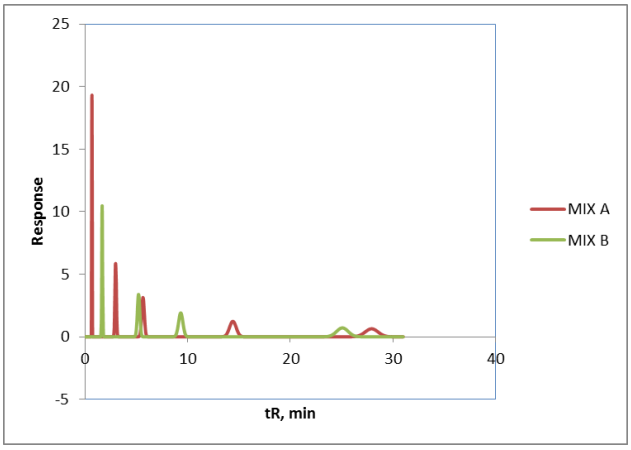
Example:

		ϕ	Predicted tR, min									
		0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1
MIX A	Thiourea	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67	0.67
	Amitriptyline	58.96	23.88	9.91	4.35	2.13	1.25	0.90	0.76	0.71	0.68	0.68
	n-Butylbenzoic acid	345.67	138.02	55.35	22.44	9.34	4.12	2.04	1.22	0.89	0.76	0.70
	Benzonitrile	125.92	50.53	20.52	8.57	3.82	1.92	1.17	0.87	0.75	0.70	0.68
	trans-Chalcone	685.16	273.17	109.15	43.86	17.86	7.51	3.40	1.75	1.10	0.84	0.74
MIX B	NN-Diethylacetamide	26.05	10.77	4.69	2.27	1.31	0.92	0.77	0.71	0.69	0.68	0.67
	Ethylbenzene	613.67	244.71	97.82	39.35	16.07	6.80	3.11	1.64	1.06	0.82	0.73
	Acetophenone	115.04	46.20	18.80	7.89	3.54	1.81	1.13	0.85	0.74	0.70	0.68
	Anisole	218.18	87.26	35.14	14.39	6.13	2.85	1.54	1.01	0.81	0.72	0.69

As you can see, the retention times at low % B is predicted to be very high (up to > 10 hours…). You can also see, that the retention times are very sensitive to small changes in % B for $0.3 \leq \phi \leq 0.5$.

Chromatogram for B = 35 %:

	tM(experimental)	0.67		Run conditions:		
	tM(manual)			% B	35	
	tM(prediction)	0.67		N	5000	
				tmax	30	
		Enter S-values here				
		S	kw	tR	w	
MIX A	Thiourea	4.000	0.0	0.67	0.04	
	Amitriptyline	4.000	87.0	2.99	0.17	
	n-Butylbenzoic acid	4.000	514.9	14.40	0.81	
	Benzonitrile	4.000	186.9	5.66	0.32	
	trans-Chalcone	4.000	1021.6	27.92	1.58	
MIX B	NN-Diethylacetamide	4.000	37.9	1.68	0.10	
	Ethylbenzene	4.000	914.9	25.07	1.42	
	Acetophenone	4.000	170.7	5.22	0.30	
	Anisole	4.000	324.6	9.33	0.53	



4) Run the experiments for LSS and calculate the results (LSS Calculations):

To determine the LSS-parameters for the compounds:

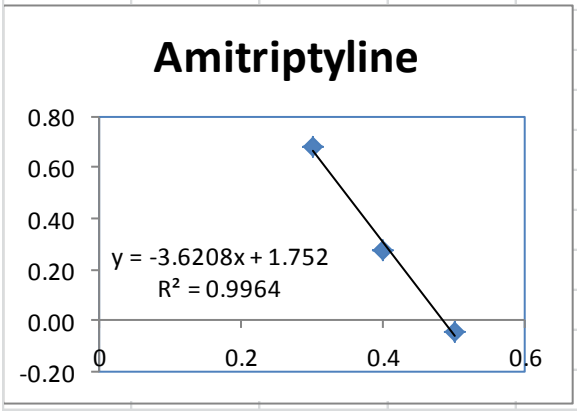
1) Run the isocratic chromatograms for several elution strengths. It is especially important to get retention times for low % B, but this may take a long time. This is important because we want to use the results for gradients, which in most cases starts at low elution strengths.
2) Enter the results in the spreadsheet. Experimental conditions in cell D4:L7, Phase ratios (0-1 for 1-100 % B) in cell D11:L11 and retention times for all compounds in cell D12:L20.
3) Look at the LSS-results in the table cell D36:F44 and the graphs below showing the linear regression of the experiments.
4) Finally you may predict retention times for other elution strengths by entering phase ratios in H34:L34 and see the retention times in H36:L44. This prediction is different from the prediction in the spreadsheet *HS Calculation*, because we now use the experimentally determined LSS-slopes instead of the default value S = 4.

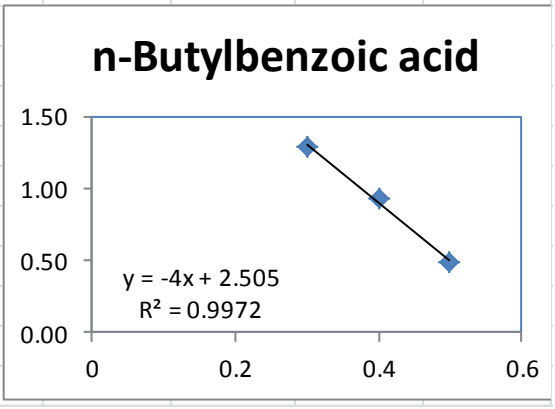
Example:

		Exprimental results:		Retention times, tR, min					Phase ratio ϕ	
	Run number	1	2	3	4	5	6	7	8	9
	Phase ratio ϕ	**0.5**	**0.4**	**0.3**						
MIX A	Thiourea	1.00	1.00	1.00						
	Amitriptyline	1.90	2.90	5.77						
	n-Butylbenzoic acid	4.11	9.50	20.62						
	Benzonitrile	2.29	4.23	9.30						
	trans-Chalcone	6.03	13.62	34.12						
MIX B	NN-Diethylacetamid	1.43	1.76	2.91						
	Ethylbenzene	6.05	13.31	31.92						
	Acetophenone	2.16	4.12	8.32						
	Anisole	3.00	6.03	14.64						

Calculated Linear-Solvent-Strength parameters log(kw) and S				
	Compound	log(kw)	S	R2
MIX A	Thiourea	NA	NA	NA
	Amitriptyline	1.752	3.621	0.99636
	n-Butylbenzoic acid	2.505	4.000	0.99721
	Benzonitrile	2.133	4.051	0.99995
	trans-Chalcone	2.745	4.094	0.99982
MIX B	NN-Diethylacetamic	1.229	3.241	0.98207
	Ethylbenzene	2.668	3.935	0.99991
	Acetophenone	2.074	4.000	0.99815
	Anisole	2.379	4.166	0.99947

Plots of log(k) vs ϕ





Calculate tR for different ϕ's:			tM	1.00
0	0.1	0.3	0.9	1
tR	tR	tR	tR	tR
1.00	1.00	1.00	1.00	1.00
57.49	25.54	5.63	1.03	1.01
320.88	128.35	21.18	1.08	1.03
136.71	54.40	9.27	1.03	1.01
557.25	217.68	33.88	1.11	1.04
17.94	9.03	2.81	1.02	1.01
466.98	189.33	31.76	1.13	1.05
119.65	48.23	8.49	1.03	1.01
240.37	92.72	14.47	1.04	1.02

The LSS-parameters log(kw) and S and/or the isocratic retention times can be used to predict the gradient retention times for the Lab Exercise in Gradient Elution using LSS or a Nonlinear Solvent Strength model.