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Bulletin 937

Use Discovery to Reduce HPLC Method Development Time

Following the Discovery method development protocol and using the Column Screening Charts (Tables 2 and 3) as a starting point can save time. It allows you to quickly identify the right column and starting conditions to achieve your separation goals. Although each method has its unique objectives for retention and selectivity, we have shown an example of how you can apply the Discovery approach to your work.

1. The Separation Objective

In this example, the goal is to separate phenacetin from codeine (structures appear in Figure A). Codeine is much less concentrated than the phenacetin, so we want codeine to elute first or at least be far enough away that the phenacetin peak doesn't interfere with the quantitation of codeine. The preferred pH is 7.0. We suspect that both compounds have impurities so we want the main peaks to be well separated.

2. Narrowing the Discovery Column Choice

The pH 7 Column Screening Chart (Table 3) shows that the compounds have the right elution order (codeine then phenacetin) on all but the Discovery HS F5 column (if the preferred elution order was reversed, the HS F5 would be the best choice!).

3. Adjusting the Starting Mobile Phase Conditions

Estimate the k' for the two compounds at the same % acetonitrile. The Column Screening Chart shows phenacetin at 20% CH_3CN and codeine at 15%. A general guideline is that k' doubles for every 5% decrease in %organic. Therefore, decreasing the % acetonitrile to 15% would double the k' of phenacetin. A concentration of 15% would be a good start. (See Table 1.)

4. Predicting Retention and Selectivity

Table 1 shows that all four phases resolved and retained the compounds. The Discovery RP-AmideC16 gave the largest selectivity. The Discovery C18 and C8 selectivity and retention were very similar. Here we would recommend doing the actual screening on three or four Discovery columns: Discovery C18 (or C8), Discovery RP-AmideC16, and Discovery Cyano.

5. Verifying the Predicted Results

In Figure A (see page 4) the sample is shown on the four Discovery columns under the mobile phase conditions predicted using the Column Screening Chart. When k' was calculated, the results were very close to predicted (see Table 1).

6. Choosing the Discovery Column for the Method

Figure A shows that the Discovery RP-AmideC16 gave the right elution order and retention, and the greatest selectivity (peak spacing). It is the best choice for this method.

Conclusions

The Discovery column screening procedure provides the analyst with several good starting points that can save significant time during method development. In this case, four Discovery columns gave the right elution order, but one of the four gave the wide peak spacing, an important requirement for this sample method. Note that each method has its unique requirements and that other Discovery columns might prove to be the best choice. The Discovery method can choose the best column no matter what the requirements of the method are.

Table 1. Predicted vs. Actual Retention

Column	Analyte	Predicted k'	Actual k'	Selectivity
Discovery C8	Codeine Phenacetin	3.6 8.2	3.6 6.7	1.86
Discovery CN	Codeine Phenacetin	1.1 2.6	1.0 1.6	1.60
Discovery RP-AmideC16	Codeine Phenacetin	3.3 9.6	2.8 8.4	3.00
Discovery C18	Codeine Phenacetin	4.4 9.4	3.3 7.7	2.33

Table 2. Guidelines for Narrowing Down the Candidate Discovery Functionalized Reversed-Phase Column for Operation at pH 2

pH 2 Operation

Use this chart as a starting point to choose one, two, three or more Discovery silica-based functionalized reversed-phase columns.

Screening Conditions:

Columns: 15cm x 4.6mm ID, 5µm particles
Mobile Phase Buffer: 25mM Phosphoric Acid, adjusted to pH 2.0 with Ammonium Hydroxide (buffer was not used in the mobile phase when non-ionic compounds were screened)
Mobile Phase Organic Modifier: CH_3CN
Flow Rate: 1mL/min
Temperature: 30°C

Note: A k' of 5 is approximately 10 minutes retention time on a 15cm x 4.6mm ID column with a flow rate of 1mL/min.

Note: For most RP-HPLC separations, assume a 2-fold decrease in k' for every 5% increase in % organic.

Compound Name	% Organic	pH	C18 k'	RP-AmideC16 k'	C8 k'	Cyano k'	HS F5 k'
5% CH_3CN							
aniline	5	pH 2	0.7	0.5	0.7	0.4	1.5
benzyl amine	5	pH 2	1.4	0.8	1.3	0.5	3.1
nizatidine	5	pH 2	1.6	1.0	1.3	0.7	2.4
o-aminobenzoic acid	5	pH 2	6.2	4.6	5.8	1.0	8.3
procainamide	5	pH 2	0.7	0.5	0.6	0.4	3.0
pyridine	5	pH 2	0.2	0.2	0.2	0.3	0.5
10% CH_3CN							
codeine	10	pH 2	2.0	1.2	1.7	0.7	2.8
hydrochlorothiazide	10	pH 2	3.0	4.3	2.7	3.1	2.3
lidocaine	10	pH 2	5.9	3.0	5.1	1.0	3.0
phentermine	10	pH 2	4.8	2.6	4.3	0.8	3.5
quinidine	10	pH 2	2.1	1.4	1.9	1.0	8.7
20% CH_3CN							
benzoic acid	20	pH 2	4.1	5.2	4.0	1.3	5.4
m-nitrobenzoic acid	20	pH 2	5.4	8.1	5.1	2.0	12.4
o-nitrobenzoic acid	20	pH 2	2.8	3.9	2.8	1.3	6.2
o-toluic acid	20	pH 2	8.4	10.3	7.8	1.8	9.7
phthalic acid	20	pH 2	1.1	1.4	1.2	0.7	2.3
p-nitrobenzoic acid	20	pH 2	6.1	9.0	5.7	2.2	15.1
sorbic acid	20	pH 2	4.1	4.3	3.8	1.1	4.5
25% CH_3CN							
acetamide	25	no buffer	0.1	0.1	0.2	0.3	0.1
anisole	25	no buffer	10.1	8.1	8.0	1.8	*
benzaldehyde	25	no buffer	3.6	3.2	3.2	1.2	4.8
benzamide	25	no buffer	0.6	0.7	0.7	0.6	1.0
benzyl alcohol	25	no buffer	1.4	1.5	1.5	0.8	1.8
methyl benzoate	25	no buffer	9.4	7.8	7.7	1.7	10.4
o-cresol	25	no buffer	4.4	6.1	4.2	1.5	5.6
phenol	25	no buffer	2.0	2.9	2.0	1.0	2.8
papaverine	25	pH 2	1.7	1.1	1.5	0.8	4.5
phenacetin	25	pH 2	2.7	3.0	2.4	1.0	1.2
30% CH_3CN							
diphenhydramine	30	pH 2	2.7	1.5	2.5	1.2	11.0
furosemide	30	pH 2	5.7	6.3	3.5	2.0	5.7
salicylic acid	30	pH 2	2.4	4.4	2.2	1.1	5.0
35% CH_3CN							
nordoxepin	35	pH 2	1.5	1.0	1.4	*	10.1
doxepin	35	pH 2	1.7	1.0	1.5	*	*
protriptyline	35	pH 2	2.5	1.6	2.1	*	*
desipramine	35	pH 2	2.5	1.5	2.1	*	*
imipramine	35	pH 2	2.8	1.5	2.4	*	13.4
nortriptyline	35	pH 2	3.0	1.8	2.6	*	12.2
amitriptyline	35	pH 2	3.4	1.9	2.9	*	14.2
trimipramine	35	pH 2	3.9	2.0	3.3	*	15.2
40% CH_3CN							
butyl paraben	40	no buffer	4.8	7.9	4.0	1.3	4.4
ethyl paraben	40	no buffer	1.4	2.5	1.4	0.8	1.9
methyl paraben	40	no buffer	0.8	1.5	0.9	0.7	1.3
propyl paraben	40	no buffer	2.6	4.4	2.4	1.0	2.9
50% CH_3CN							
bromobenzene	50	no buffer	3.8	3.2	2.8	1.0	3.2
chlorobenzene	50	no buffer	3.3	2.8	2.5	1.0	3.0
fluorobenzene	50	no buffer	2.0	1.8	1.7	0.8	2.3
nitrobenzene	50	no buffer	1.4	1.4	1.3	0.8	1.9
nitrosobenzene	50	no buffer	1.6	1.6	1.5	0.8	2.1
fluoxetine	50	pH 2	2.1	1.2	0.8	0.6	13.4
ibuprofen	50	pH 2	4.3	4.9	3.4	1.0	2.9
norfluoxetine	50	pH 2	1.8	1.2	0.7	0.6	11.1
55% CH_3CN							
1,3,5-tribromobenzene	55	no buffer	13.0	9.4	6.0	1.1	5.0
1,3-dinitrobenzene	55	no buffer	1.0	1.0	1.0	0.7	1.5
1-chloro-2-fluorobenzene	55	no buffer	2.3	2.1	1.9	0.7	2.3
2-chloronitrobenzene	55	no buffer	1.4	1.4	1.3	0.7	1.9
4-bromochlorobenzene	55	no buffer	4.5	3.8	2.9	0.9	3.1
4-nitrophenol	55	no buffer	0.5	1.0	0.6	0.5	0.8
hexafluorobenzene	55	no buffer	2.6	2.1	2.2	0.7	3.1
pentachlorobenzene	55	no buffer	18.1	12.4	8.0	1.3	7.5
60% CH_3CN							
benzene	60	no buffer	1.2	1.0	1.1	0.6	1.2
butyl benzene	60	no buffer	6.4	4.4	3.9	0.8	3.2
ethyl benzene	60	no buffer	2.6	2.1	1.9	0.7	1.9
propyl benzene	60	no buffer	4.1	3.0	2.7	0.7	2.5
toluene	60	no buffer	1.8	1.5	1.4	0.6	1.6

* meaningful data could not be obtained due to coelution or other problem

Table 3. Guidelines for Narrowing Down the Candidate Discovery Functionalized Reversed-Phase Column for Operation at pH 7

pH 7 Operation

Use this chart as a starting point to choose one, two, three or more Discovery silica-based functionalized reversed-phase columns.

Screening Conditions:

Columns: 15cm x 4.6mm ID, 5µm particles

Mobile Phase Buffer: 25mM Phosphoric Acid, adjusted to pH 7 with Ammonium Hydroxide (buffer was not used in the mobile phase when non-ionic compounds were screened)

Mobile Phase Organic Modifier: CH_3CN

Flow Rate: 1mL/min

Temperature: 30°C

Note: A k' of 5 is approximately 10 minutes retention time on a 15cm x 4.6mm ID column with a flow rate of 1mL/min.

Note: For most RP-HPLC separations, assume a 2-fold decrease in k' for every 5% increase in % organic.

Compound Name	% Organic	pH	C18 k'	RP-AmideC16 k'	C8 k'	Cyano k'	HS F5 k'
5% CH_3CN							
aniline	5	pH 7	7.1	4.4	6.6	1.3	8.6
benzoic acid	5	pH 7	1.4	1.1	1.5	*	2.4
benzyl amine	5	pH 7	1.5	1.2	1.4	0.7	6.7
m-nitrobenzoic acid	5	pH 7	3.5	3.0	*	1.0	10.2
o-aminobenzoic acid	5	pH 7	1.2	1.0	1.2	0.5	0.4
o-nitrobenzoic acid	5	pH 7	1.0	0.7	1.0	*	0.9
o-toluic acid	5	pH 7	1.7	1.2	1.8	*	2.0
phthalic acid	5	pH 7	0.1	0.2	0.3	0.2	0.0
p-nitrobenzoic acid	5	pH 7	3.2	3.1	*	1.1	*
procainamide	5	pH 7	3.0	2.4	2.4	1.0	2.4
pyridine	5	pH 7	3.5	2.3	3.5	0.9	5.6
sorbic acid	5	pH 7	1.8	1.3	1.9	*	2.6
10% CH_3CN							
hydrochlorothiazide	10	pH 7	3.0	4.2	2.7	3.0	1.9
nizatidine	10	pH 7	6.1	4.3	4.9	1.2	7.4
phentermine	10	pH 7	5.3	4.0	4.8	1.3	3.8
15% CH_3CN							
codeine	15	pH 7	4.4	3.3	3.6	1.1	3.0
20% CH_3CN							
phenacetin	20	pH 7	4.7	4.8	4.1	1.3	2.2
25% CH_3CN							
acetamide	25	no buffer	0.1	0.1	0.2	0.3	0.1
anisole	25	no buffer	10.1	8.1	8.0	1.8	*
benzaldehyde	25	no buffer	3.6	3.2	3.2	1.2	4.8
benzamide	25	no buffer	0.6	0.7	0.7	0.6	1.0
benzyl alcohol	25	no buffer	1.4	1.5	1.5	0.8	1.8
methyl benzoate	25	no buffer	9.4	7.8	7.7	1.7	10.4
o-cresol	25	no buffer	4.4	6.1	4.2	1.5	5.6
phenol	25	no buffer	2.0	2.9	2.0	1.0	2.8
furosemide	25	pH 7	1.8	1.7	1.7	1.0	1.3
salicylic acid	25	pH 7	0.4	0.4	0.5	0.5	1.0
30% CH_3CN							
papaverine	30	pH 7	5.9	5.8	4.9	1.7	2.9
quinidine	30	pH 7	1.5	2.2	1.4	1.3	5.0
40% CH_3CN							
butyl paraben	40	no buffer	4.8	7.9	4.0	1.3	4.4
ethyl paraben	40	no buffer	1.4	2.5	1.4	0.8	1.9
methyl paraben	40	no buffer	0.8	1.5	0.9	0.7	1.3
propyl paraben	40	no buffer	2.6	4.4	2.4	1.0	2.9
diphenhydramine	40	pH 7	2.0	1.9	1.9	1.6	6.8
fluoxetine	40	pH 7	2.6	3.4	2.6	2.4	9.0
ibuprofen	40	pH 7	0.8	0.8	0.9	0.5	1.7
lidocaine	40	pH 7	4.4	3.6	3.3	1.1	3.0
norfluoxetine	40	pH 7	2.1	3.3	2.1	2.0	6.4
50% CH_3CN							
bromobenzene	50	no buffer	3.8	3.2	2.8	1.0	3.2
chlorobenzene	50	no buffer	3.3	2.8	2.5	1.0	3.0
fluorobenzene	50	no buffer	2.0	1.8	1.7	0.8	2.3
nitrobenzene	50	no buffer	1.4	1.4	1.3	0.8	1.9
nitrosobenzene	50	no buffer	1.6	1.6	1.5	0.8	2.1
55% CH_3CN							
1,3,5-tribromobenzene	55	no buffer	13.0	9.4	6.0	1.1	5.0
1,3-dinitrobenzene	55	no buffer	1.0	1.0	1.0	0.7	1.5
1-chloro-2-fluorobenzene	55	no buffer	2.3	2.1	1.9	0.7	2.3
2-chloronitrobenzene	55	no buffer	1.4	1.4	1.3	0.7	1.9
4-bromochlorobenzene	55	no buffer	4.5	3.8	2.9	0.9	3.1
4-nitrophenol	55	no buffer	0.5	1.0	0.6	0.5	0.8
hexafluorobenzene	55	no buffer	2.6	2.1	2.2	0.7	3.1
pentachlorobenzene	55	no buffer	18.1	12.4	8.0	1.3	7.5
amitriptyline	55	pH 7	2.0	1.7	1.8	*	8.4
doxepin	55	pH 7	1.2	1.1	1.2	*	7.8
imipramine	55	pH 7	1.4	1.3	1.4	*	8.4
nordoxepin	55	pH 7	0.4	0.6	0.5	*	6.3
nortriptyline	55	pH 7	0.6	1.0	0.7	*	7.6
protriptyline, desipramine	55	pH 7	0.5	0.8	0.6	*	6.3
trimipramine	55	pH 7	3.0	2.3	2.2	*	9.1
60% CH_3CN							
benzene	60	no buffer	1.2	1.0	1.1	0.6	1.2
butyl benzene	60	no buffer	6.4	4.4	3.9	0.8	3.2
ethyl benzene	60	no buffer	2.6	2.1	1.9	0.7	1.9
propyl benzene	60	no buffer	4.1	3.0	2.7	0.7	2.5
toluene	60	no buffer	1.8	1.5	1.4	0.6	1.6

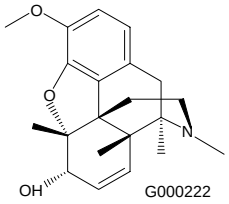
* meaningful data could not be obtained due to coelution or other problem

Figure A. Chromatograms from Column Screening

Columns: **15cm x 4.6mm ID, 5µm particles**
Mobile Phase: 85:15, 25mM H_3PO_4, pH 7.0 w/NH_4OH:CH_3CN
Flow Rate: 1mL/min
Detection: UV, 220nm
Inj.: 10µLm each compound 0.1mg/mL

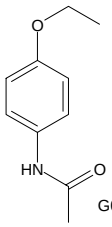
1. Codeine
2. Phenacetin

Codeine



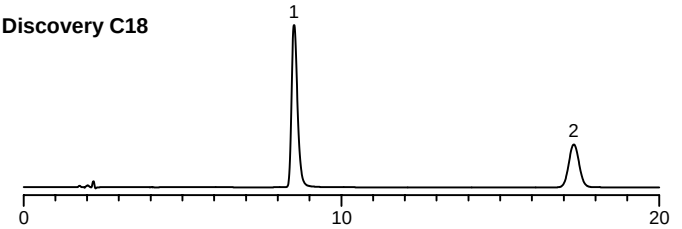
G000222

Phenacetin



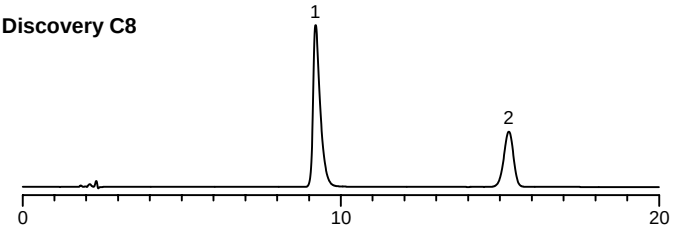
G002061

Discovery C18



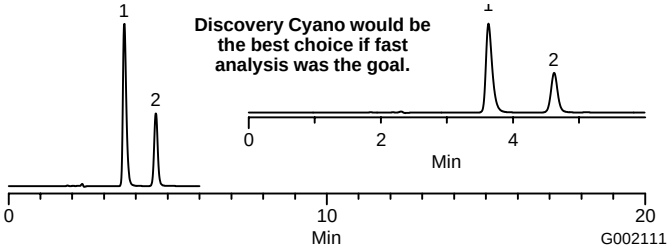
G002109

Discovery C8



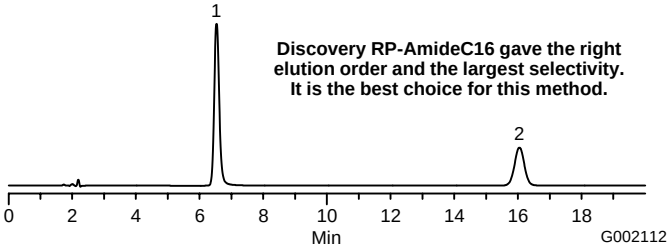
G002110

Discovery Cyano



G002111

Discovery RP-AmideC16



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Ordering Information

Other dimensions and Discovery phases are available. Please call or visit our web site.

Phase	Cat. No.
Columns: 15cm x 4.6mm ID, 5µm particles	
Discovery C18	**504955**
Discovery C8	**59353-U**
Discovery Cyano	**59356-U**
Discovery RP-AmideC16	**505013**
Discovery HS F5	**59356-U**
Discovery HS PEG	**505013**

Phase	Pack of 2 Cat. No.	Kit[2] Cat. No.
Supelguard Cartridges: 2.0cm x 4.0mm ID, 5µm particles		
Discovery C18[1]	**505137**	**505129**
Discovery C8[1]	**59590-U**	**59589-U**
Discovery Cyano[1]	**59585-U**	**59586-U**
Discovery RP-AmideC16[1]	**505099**	**505080**
Discovery HS F5[1]	**567576-U**	**567577-U**
Discovery HS PEG[1]	**567476-U**	**567477-U**

[1]For 4.0mm ID and 4.6mm ID analytical columns.
[2]Kits include one cartridge, a stand-alone holder, a piece of tubing, and 2 nuts and ferrules.

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