Benzoate SFC Separations on Chiral and Achiral Stationary Phases

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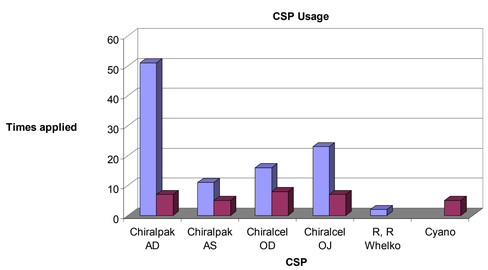
Introduction

Our laboratory has supported Drug Discovery by using preparative SFC in isolating optically pure enantiomers for in-vitro and in-vivo assays. Recently more of the work of the group has turned toward applying SFC to chemical purifications to meet the high purity standards needed for in-vivo testing when the usual purification methods are ineffective in achieving those standards. Preparative SFC has been used in these instances to insure timely deliveries of the API to Pharmaceutics and Biologists for the scheduled tests.

Often these impurities differ from the parent compound either as a regio-isomer, or by halogen substitution, or by differences in methyl substitution. Our group has relied heavily on polysaccharide coated stationary phases (CSP's) used in chiral separations for their removal. Because of the expense of chiral columns it seemed worth exploring the use of other achiral columns for this type of work. A preliminary experiment was designed screening test mixtures of methyl and ethyl benzoates containing various substituents by SFC, comparing separations on sets of polysaccharide columns we normally use to some achiral columns of interest. This presentation will discuss those results.

Column Use for SFC Preparative Separations

This graph illustrates the frequency of column use for the preparative separation of 103 different racemic mixtures in blue and 32 different mixtures requiring achiral purification in purple.



- Notice the prevalent use of AD for chiral resolutions and a more even distribution of the Daicel polysaccharide columns for achiral work. Preparative DIOL and silica columns were also available but underperformed the cyano in the screening of the 32 compounds requiring purification.

Analytical Conditions

These columns and conditions were used in the screening. The chiral columns selected were those used most frequently. The choice of achiral columns are available in large scale for preparative work and recommended by colleagues.

Chiral Technology Chiral Columns:

Chiralpak AD-H 4.6x250 mm 5µ
Chiralpak AS-H 4.6x250 mm 5µ
Chiralcel OD-H 4.6x250 mm 5µ
Chiralcel OJ-H 4.6x250 mm 5µ

Princeton Chromatography Achiral Columns:

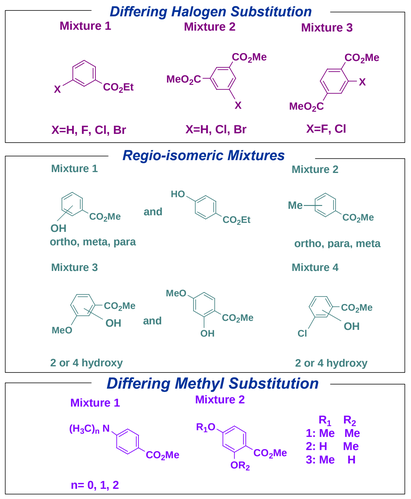
DIOL HL 4.6x250 mm 5µ
2CN:DIOL 4.6x250 mm 5µ
2-Ethylpyridine 4.6x250 mm 5µ
Cyano 4.6x250 mm 5µ

Separation Method:

Thar Analytical SFC and SFC/MS
Mobile Phase: 10% methanol in CO_2
Flow rate: 3 ml/min
Temperature: 35°C
Pressure: 100 bar
Detection: UV 190-400 nm
Injection: 5-10µl

Test Mixtures

Looking for trends in compound retention of methyl and ethyl benzoates with variations in aryl halogen substitution, methyl substitution, and comparing regio-isomers, the following compound mixtures were screened by SFC on the columns of interest. These compounds are commercially available from Sigma Aldrich and Alfa Aesar and were supplied by our central chemical storage facility.

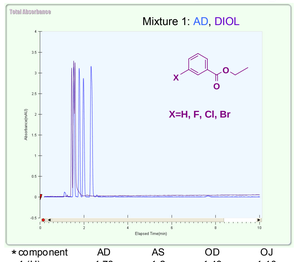


Results

*The chromatograms display the best and worst of the separations for each sample mixture listing columns in the order of their effectiveness in separating components.

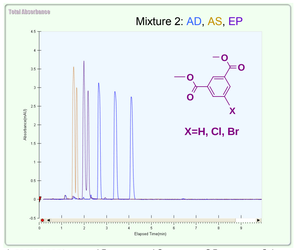
Below the chromatograms individual tables denote retention times of the various solutes on all of the columns screened using the above described separation conditions. Because of the solutes' very short retention times in the case of regio-isomer mixture 2, it was necessary to run at 2ml/min with 3% modifier.

Screening of Halogen Substituted Mixtures



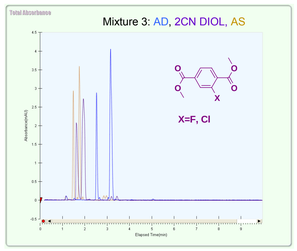
* component	AD	AS	OD	OJ
1 (H)	1.78	1.2	1.48	1.40
2 (F)	1.52	to	1.69	1.7
3 (Cl)	1.97	1.4	1.84	1.7
4 (Br)	2.32	1.46	2.12	1.88

	cyano	EP	DIOL HL	2CN DIOL
1 (H)	1.3	1.3	1.45	1.43
2 (F)		to	1.5	1.5
3 (Cl)	to	1.80	1.5	1.5
4 (Br)	1.9	1.87	1.65	1.59



* component	AD	AS	OD	OJ
1 (H)	2.65	1.5	2.37	1.89
2 (Cl)	3.37	1.5	2.53	2.2
3 (Br)	4.10	1.67	2.99	2.2

	cyano	EP	DIOL HL	2CN DIOL
1 (H)	1.6	2.0	1.57	1.64
2 (Cl)	to	2.0	1.7 to	1.7 to
3 (Br)	2	2.17	2	2



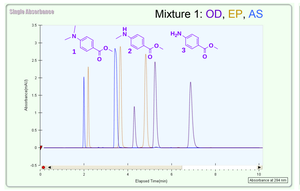
*component	AD	AS	OD	OJ
1 (F)	2.52	1.48	2.10	1.91
2 (Cl)	3.14	1.75	2.70	2.60

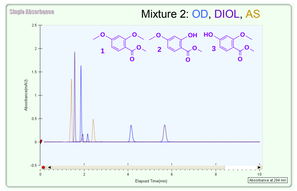
	cyano	EP	DIOL HL	2CN DIOL
1 (F)	1.76	1.80	1.56	1.62
2 (Cl)	2.06	2.18	1.83	1.93

- In the halogen series the AD and OD columns prove to be the most effective in separating all components. Elution order on all columns is typically in order of MW.

Results (cont'd)

Screening of Mixtures With Varying Methyl Substitution





* component	AD	AS	OD	OJ
1 (NMe_2)	5.08	1.99	4.29	5.34
2 (NHMe)	6.05	3.4	5.24	5.66
3 (NH_2)	7.09	3.4	6.86	6.90

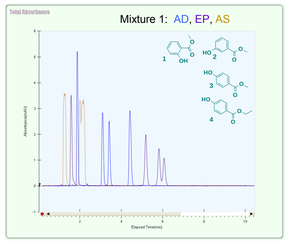
	cyano	EP	DIOL HL	2CN DIOL
1 (NMe_2)	2.14	2.19	2.16	2.14
2 (NHMe)	3.03	3.65	3.59	3.11
3 (NH_2)	3.70	4.82	5.11	4.02

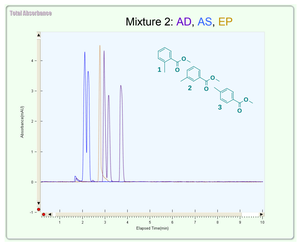
*component	AD	AS	OD	OJ
1 (3Me)	2.28	1.4	1.85	2.0
2 (p-OMe)	2.47	1.4	2.16	2.0
3 (o-OMe)	3.97	2.41	4.14	3.03

	cyano	EP	DIOL HL	2CN DIOL
1 (3Me)	1.59	1.72	1.57	1.56
2 (p-OMe)	1.95	1.93	1.94	1.97
3 (o-OMe)	3.71	7.02	5.68	4.25

- Here we see good separation of all components for each mixture on all of the achiral columns and some of the chiral columns with the AS giving the poorest separation in both cases. In mixture 2, the separation of 1 and 2 are equally poor on OJ and AS. It appears the achiral columns are more sensitive to the differences in polarity of the solutes.

Screening of Regio-Isomer Mixtures





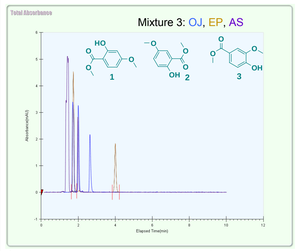
* component	AD	AS	OD	OJ
1 (o-OH))	1.85	1.25	1.56	1.63
2 (m-OH)	4.41	1.9	2.9	2.74
3 (p-OH)	3.36	to	3.17	2.95
4 Et (p-OH)	3.04	2.4	2.9	3.1

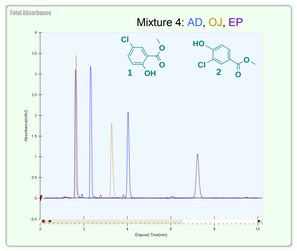
	cyano	EP	DIOL HL	2CN DIOL
1 (o-OH))	1.5	1.6	1.46	1.46
4 Et (p-OH)	2.79	5.19	3.83	2.95
2 (m-OH)	2.94	5.82	4.09	3.11
3 (p-OH)	3.1	6.10	4.30	3.2

*component	AD	AS	OD	OJ
1	2.96	2.16	3.19	3.03
2	3.16	2.16	3.30	3.21
3	3.70	2.31	3.75	3.50

	cyano	EP	DIOL HL	2CN DIOL
1	2.60	1.90	2.73	2.63
2	2.60	1.90	2.73	2.63
3	2.65	1.90	2.80	2.71

- Ethyl benzoate 4 is not a regio-isomer but was added in mixture 1 to compare its retention to the methyl benzoate 3. Interestingly 4 preceded 3 in elution order in most cases both on the chiral and achiral columns and preceded the m-OH methyl benzoate, 2, on all of the achiral columns.





*component	AD	AS	OD	OJ
1 (m-OMe)	2.49	1.4	1.8	1.71
2 (p-OMe)	2.74	1.4	1.8	2.00
3 (p-OH))	3.54	1.98	3.11	2.63

	cyano	EP	DIOL HL	2CN DIOL
1 (m-OMe)	1.62	1.73	1.6	1.60
2 (p-OMe)	1.62	1.73	1.6	1.60
3 (p-OH)	2.62	4.00	3.33	2.75

*component	AD	AS	OD	OJ
1 (o-OH)	2.31	1.37	1.73	1.64
2 (p-OH)	4.03	2.27	3.44	3.28

	cyano	EP	DIOL HL	2CN DIOL
1 (o-OH)	1.48	1.62	1.47	1.46
2 (p-OH)	3.01	7.22	4.3	3.17

- In the regio-isomer series, the AD and OJ columns seem to be the most reliable in separating all components. The greatest difference between retentions of the o-OH and p-OH benzoates are seen with the EP column. Again the AS column shows poorest separation.

Conclusions

For this benzoate series of compounds:

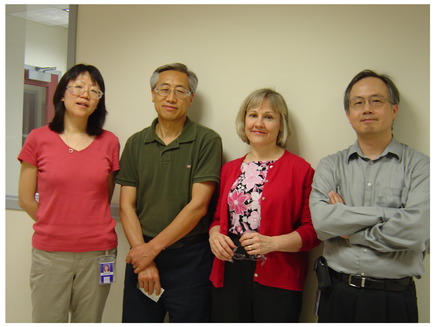
- With variation in halogen substitution, the chiral columns outperformed the achiral columns. Separation of all components are seen on the AD and OD. The elution order is generally in order of molecular weight on all stationary phases.
- The achiral columns responded better to differences in the solutes' polarities than the chiral columns. Of the achiral columns examined here the EP offered greater separation possibilities than the others. It showed the greatest differentiation between the ortho- and para-hydroxy substituted benzoates.
- The AS column gave poorest resolution in most cases and underperformed the achiral columns in many cases when better separation was seen with other polysaccharide columns.

Summary – The Take Home Message

One bears in mind the problems associated with applying the results of this experiment to larger compounds containing many other functionalities that interact both with the mobile phase as well as the stationary phase. Screening in each application is a must. It is helpful in seeing certain correlations, though, when considering future purchases of equipment. Here, the AS column probably underperforms because these non-polar benzoates aren't very well retained. Generally of the polysaccharide columns, compounds tend to elute more quickly on the AS column. We have done separation work where the AS column has been the only one in which a separation could be attained. We've used it also in situations when the separation on other columns was too great and reducing the retention times of the solutes made setting injection intervals in preparative work practical. It may not get frequent use but it has been invaluable to getting our work done. The wider range of tools available to the chromatographer, the greater the ability to accomplish the tasks at hand. With limited resources one needs to make an educated guess as to the best tools to add to the tool box. This experiment would suggest these chiral columns serve as well or better than many achiral columns in compound purification. Considering cost, it is wisest to use the less expensive tools when possible and save the more expensive ones for applications where they are necessary. As far as achiral column choices go, the EP column looks like an attractive alternative to add to our tool box.

Acknowledgements

DCS Separations Group:



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• Balu Balasubramanian (not shown)

Photograph provided by Sarah Traeger