Evaluation of amylose and cellulose based chiral stationary phases from different vendors using supercritical fluid chromatography

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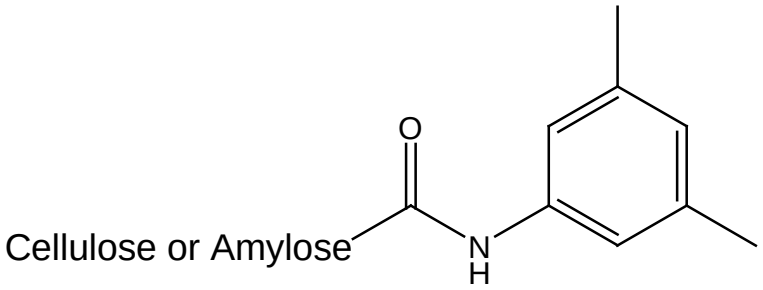
Outline

- Overview
- Evaluation of Amylose based chiral stationary phases
- Evaluation of Cellulose based chiral stationary phases
- Conclusions

Overview

- Chiral separation in pharmaceutical industry
- SFC for chiral method development
- Amylose and cellulose based chiral stationary phases
- Tris-(3,5-dimethylphenyl) carbamoyl amylose and Tris-(3,5-dimethylphenyl) carbamoyl cellulose are two major chiral stationary phases (CSP)



Overview, continued

- Columns have become available from several vendors that have the same stationary phase. This work was performed to evaluate such chiral columns using a column screening platform and supercritical fluid chromatography (SFC).
- Columns were selected from five vendors: Akzo Nobel, Chiral Technologies, Macherey-Nagel, Phenomenex, and Regis Technologies.
- Two sets of comparisons were completed to evaluate amylose and cellulose-based columns. Five representative racemic compounds were used for each set of comparisons to evaluate column performance for both selectivity and resolution.

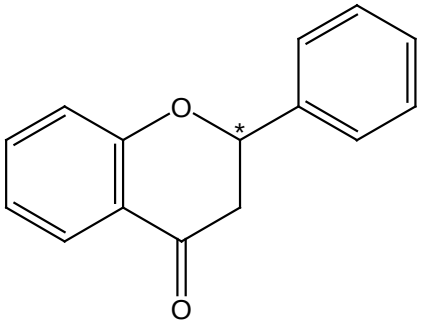
Overview, continued

- Four factors were considered for comparison:
  - Enantioselectivity (α)
  - Resolution (Rs)
  - Peak Height
  - Retention time (RT)

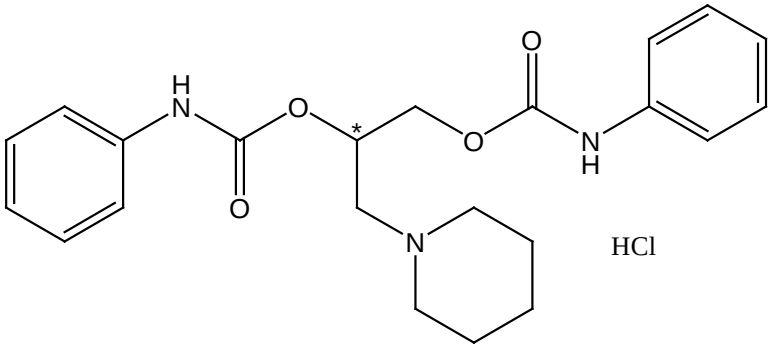
Comparison of tris-(3,5-dimethylphenyl) carbamoyl amylose chiral stationary phases

- Chiralpak AD-H (250 × 4.6 mm, 5 μm)
- ChiralPak IA (250 × 4.6 mm, 5 μm)
- Kromasil 3-AmyCoat (250 × 4.6 mm, 3 μm)
- Macherey-Nagel Nucleocel Alpha S (250 × 4.6 mm, 5 μm)
- RegisPack (250 × 4.6 mm, 5 μm)

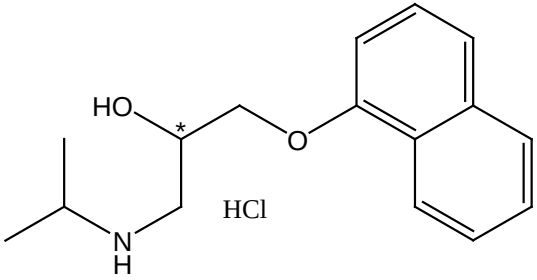
Structures of five chiral compounds



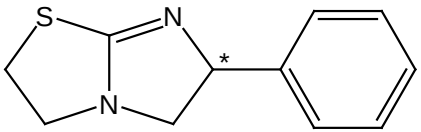
Flavanone
Aldrich Cat. #102032



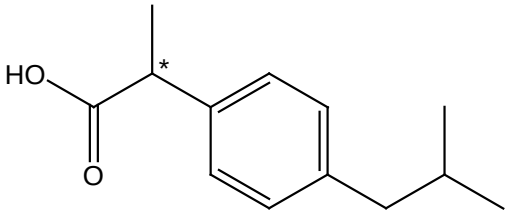
Diperodon hydrochloride
Fluka Cat. #D8536



Propranolol hydrochloride
Sigma Cat. #P0884



Tetramisole
Sigma Cat. #L9756

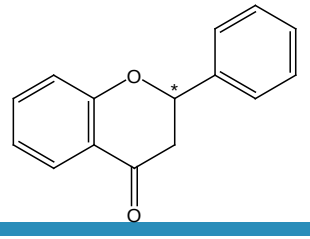


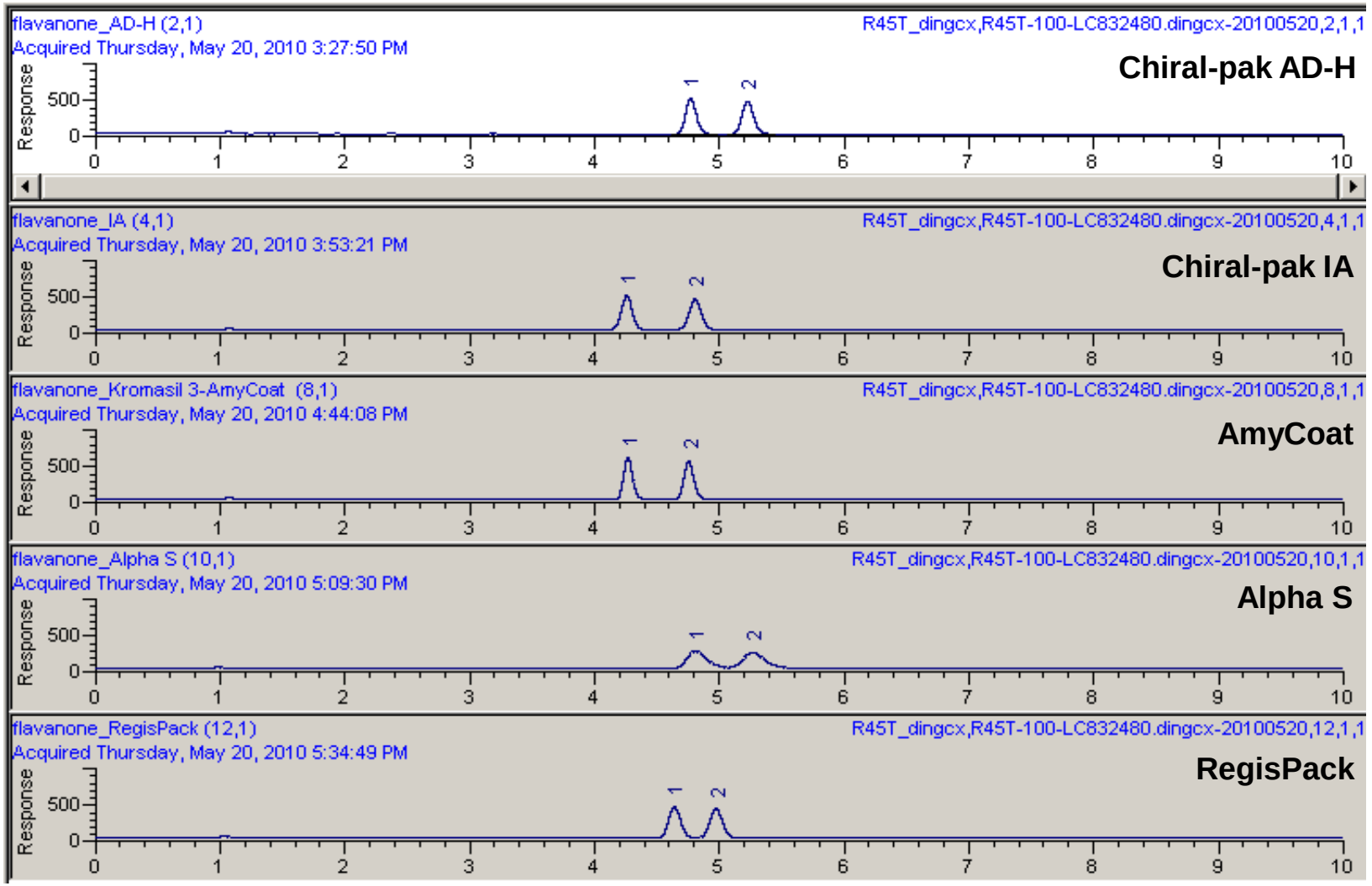
Ibuprofen
Sigma Cat. #I4883

SFC Conditions

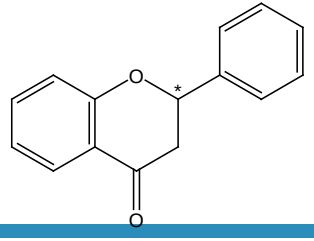
- Berger SFC system with column and modifier switching systems
- Column temperature: 35 °C
- Evaporator temperature: 27 °C
- Trimmer temperature: 27 °C
- Flow rate: 3.0 mL/min
- Pressure: 100 bar
- UV detection: 210 nm
- Injection volume: 10 μL
- Software: SFC ProNTo and Thermo Atlas

Flavanone, 10% IPA, 90%CO_2



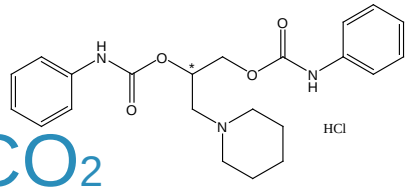


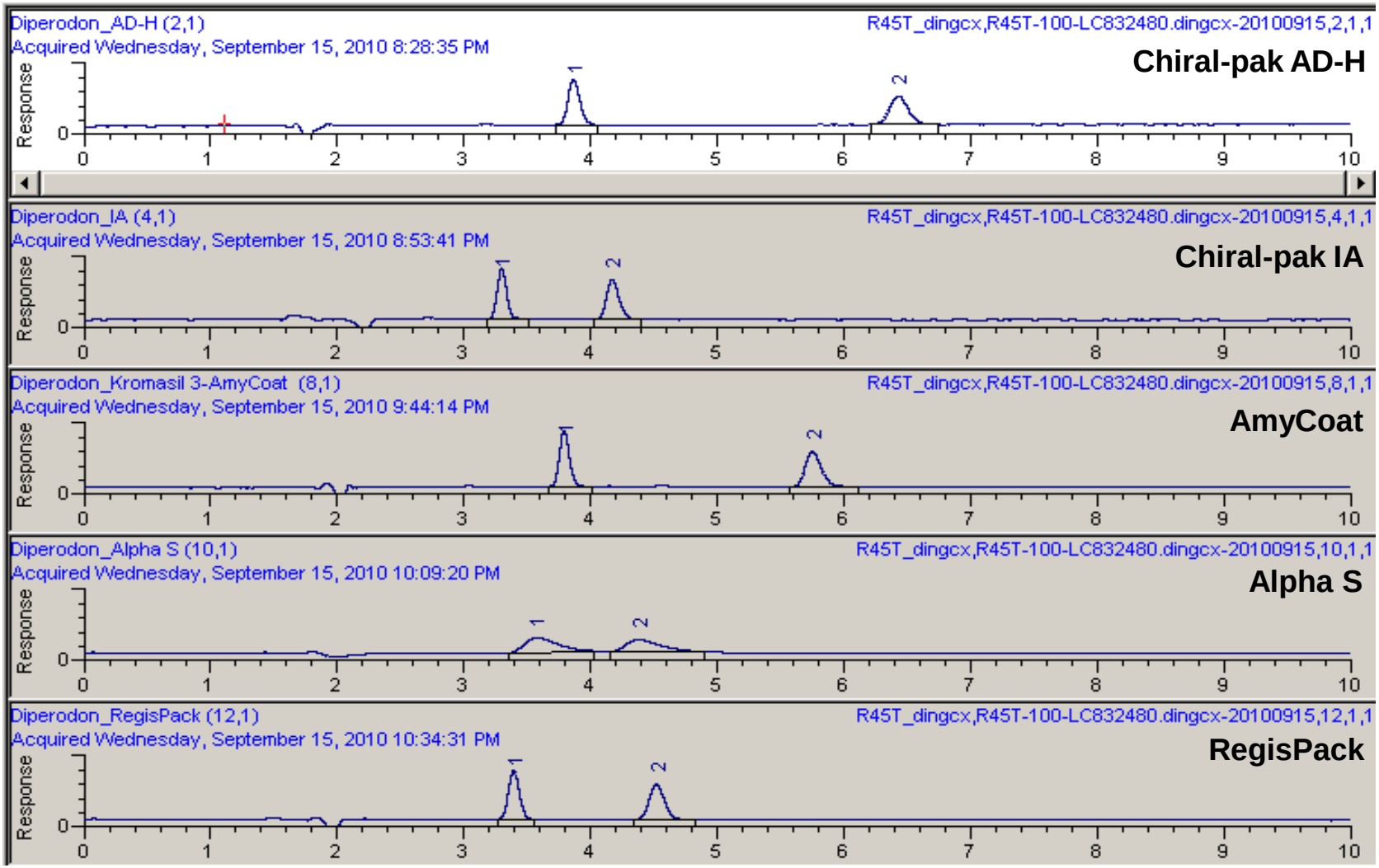
Flavanone



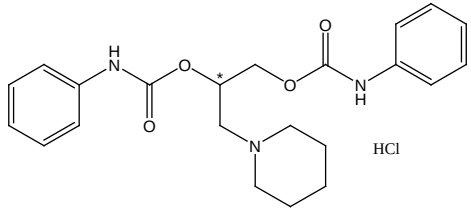
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak AD-H	1.11	2.7	493/454	4.77/5.23
Chiralpak IA	1.15	3.2	476/423	4.26/4.81
Kromasil 3-AmyCoat	1.15	3.5	577/523	4.27/4.76
Macherey-Nagel Nucleocel Alpha S	1.13	1.4	239/220	4.81/5.27
RegisPack	1.11	1.8	426/398	4.63/4.98

Diperodon, 30% EtOH w/ 0.1% TEA, 70%CO_2



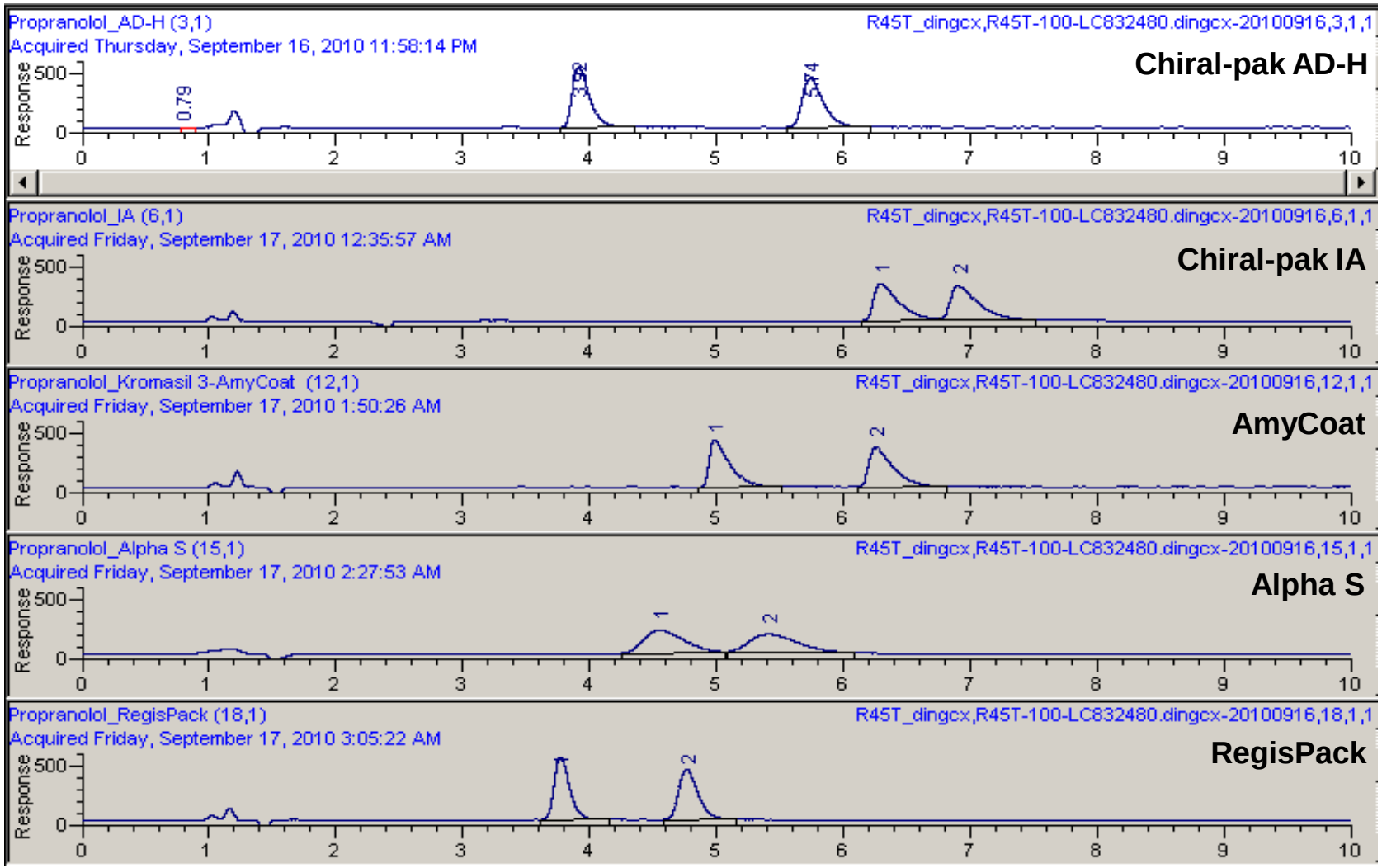


Diperodon

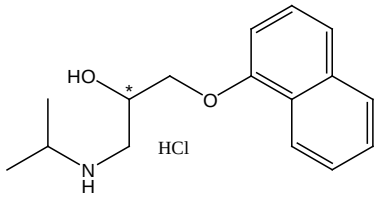


	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak AD-H	1.86	11.7	318/199	3.87/6.43
Chiralpak IA	1.39	5.4	352/275	3.30/4.17
Kromasil 3-AmyCoat	1.71	10.3	385/247	3.79/5.75
Macherey-Nagel Nucleocel Alpha S	1.31	1.6	101/86	3.57/4.38
RegisPack	1.46	5.9	336/247	3.40/4.52

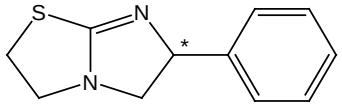
Propranolol, 15% MeOH w/ 0.1% TEA, 85%CO_2



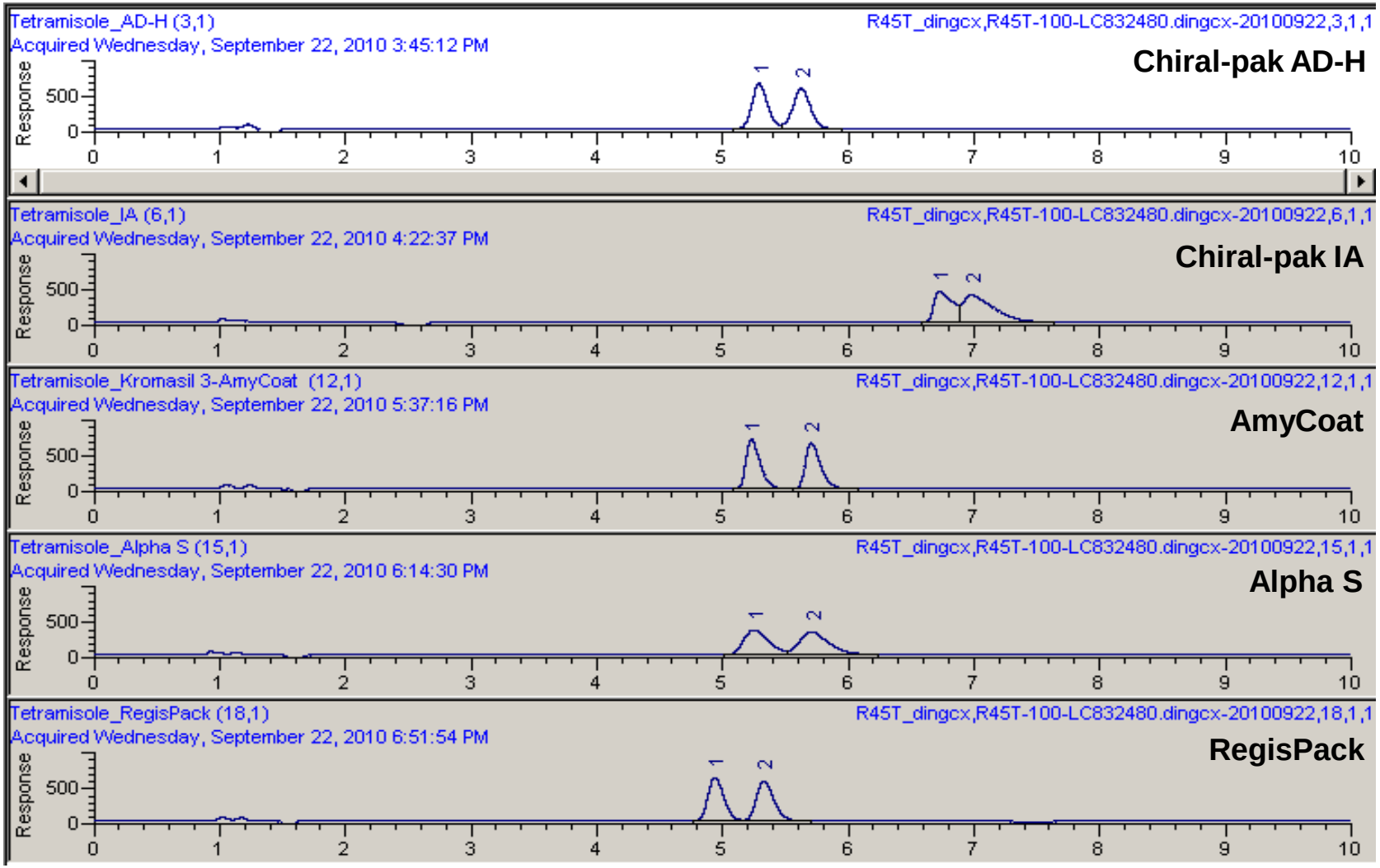
Propranolol



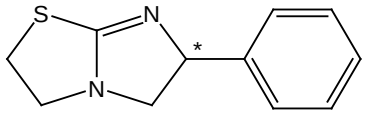
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak AD-H	1.62	6.6	501/408	3.92/5.74
Chiralpak IA	1.11	1.6	313/285	6.30/6.91
Kromasil 3-AmyCoat	1.33	3.9	394/328	4.99/6.26
Macherey-Nagel Nucleocel Alpha S	1.22	1.3	186/148	4.55/5.42
RegisPack	1.36	4.0	524/421	3.77/4.77



Tetramisole, 13% MeOH w/ 0.1% TEA, 87%CO_2

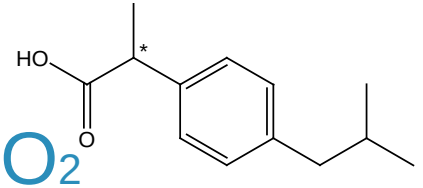


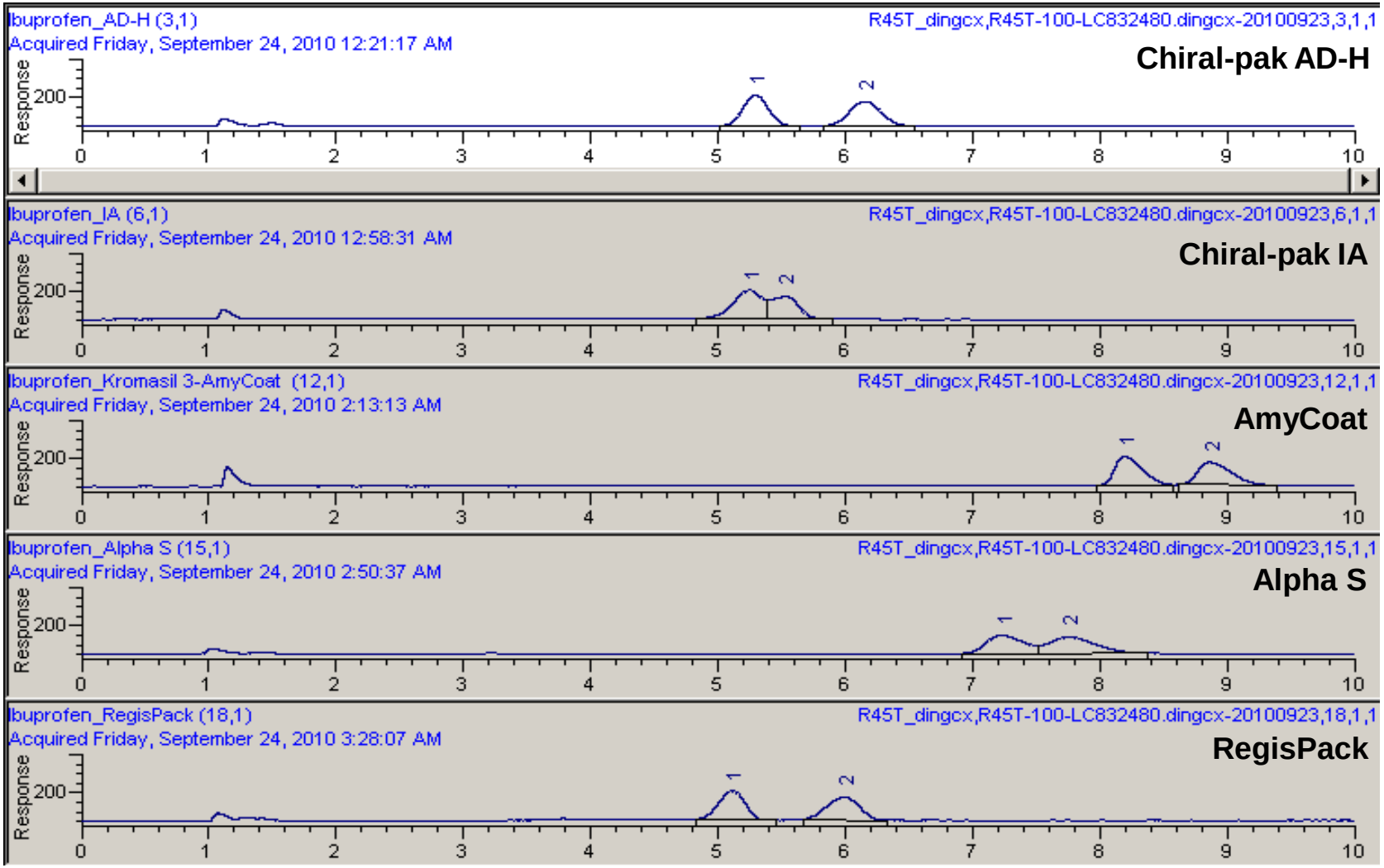
Tetramisole



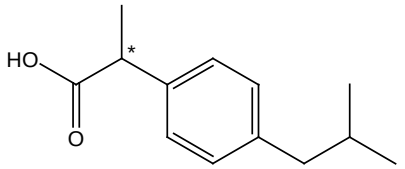
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak AD-H	1.07	1.4	631/560	5.30/5.63
Chiralpak IA	1.05	NA	425/374	6.73/6.98
Kromasil 3-AmyCoat	1.12	2.2	686/631	5.23/5.71
Macherey-Nagel Nucleocel Alpha S	1.09	1.1	344/313	5.26/5.71
RegisPack	1.10	1.6	603/550	4.94/5.33

Ibuprofen, 3% MeOH w/ 0.1% ESA, 97%CO_2





Ibuprofen



	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak AD-H	1.21	2.1	160/128	5.30/6.16
Chiralpak IA	1.05	NA	151/118	5.25/5.53
Kromasil 3-AmyCoat	1.10	1.5	152/118	8.20/8.87
Macherey-Nagel Nucleocel Alpha S	1.10	0.7	95/84	7.24/7.76
RegisPack	1.22	2.0	149/118	5.12/5.99

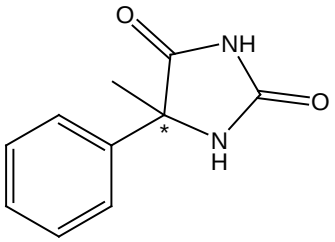
Conclusion for amylose based columns

- Kromasil 3-AmyCoat generally produced good chiral separations and the highest peak height among the columns studied.
- Chiralpak AD-H produced good chiral separations for each of the compounds.
- Although some of the separations were good, Chiralpak IA did not give comparable chiral separations to the AD-H for the compounds studied.
- RegisPack had similar performance to the Chiralpak AD-H column for most of the compounds studied.

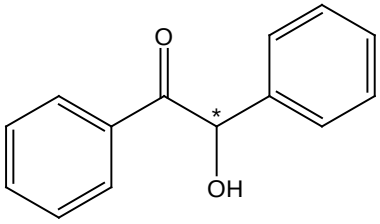
Comparison of tris-(3,5-dimethylphenyl) carbamoyl cellulose chiral stationary phases

- Chiralcel OD-H (250 × 4.6 mm, 5 μm)
- ChiralPak IB (250 × 4.6 mm, 5 μm)
- Kromasil 3-CelluCoat (250 × 4.6 mm, 3 μm)
- Phenomenex Lux Cellulose-1 (250 × 4.6 mm, 3 μm)
- Macherey-Nagel Nucleocel Delta S (250 × 4.6 mm, 5 μm)
- RegisCell (250 × 4.6 mm, 5 μm)

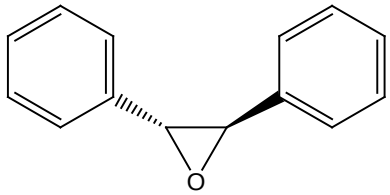
Structures of five chiral compounds



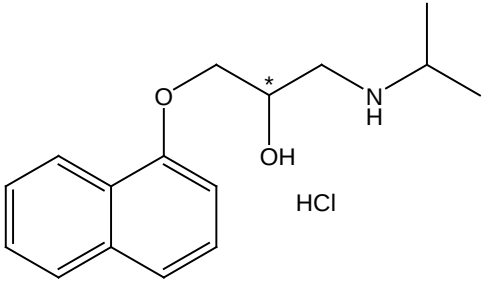
5-Methyl-5-phenylhydantoin
Aldrich, Cat. #180823



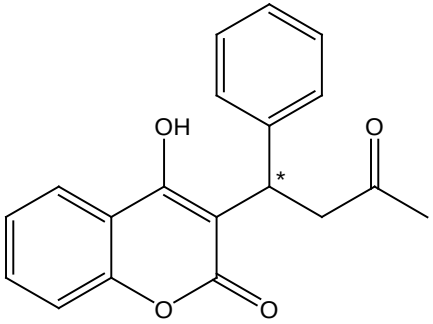
Benzoin
Aldrich, Cat. #8681



trans-Stilbene oxide
Aldrich, Cat. #S4921



Propranolol hydrochloride
Sigma, Cat. #P0884

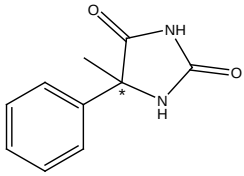


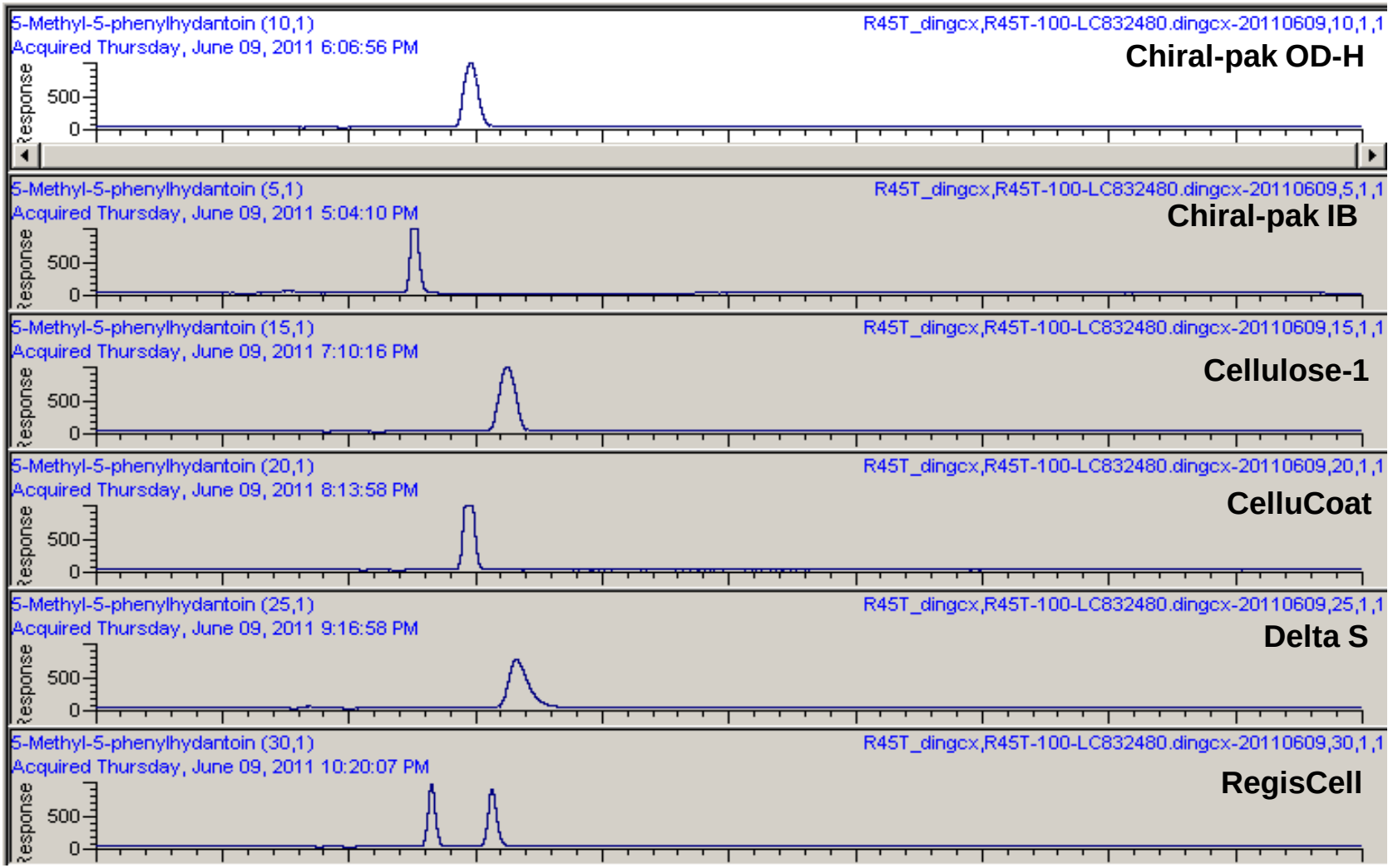
Warfarin
Fluka, Cat. #A2250

SFC Conditions

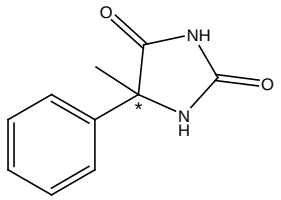
- Berger SFC system with column and modifier switching systems
- Column temperature: 35 °C
- Evaporator temperature: 27 °C
- Trimmer temperature: 27 °C
- Flow rate: 3.0 mL/min
- Pressure: 100 bar
- UV detection: 210 nm
- Injection volume: 10 μL
- Software: SFC ProNTo and Thermo Atlas

5-Methyl-5-phenylhydantoin, 25% IPA, 75%CO_2



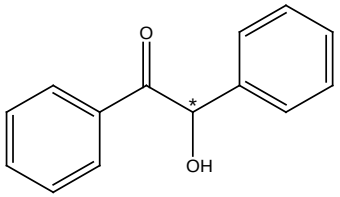


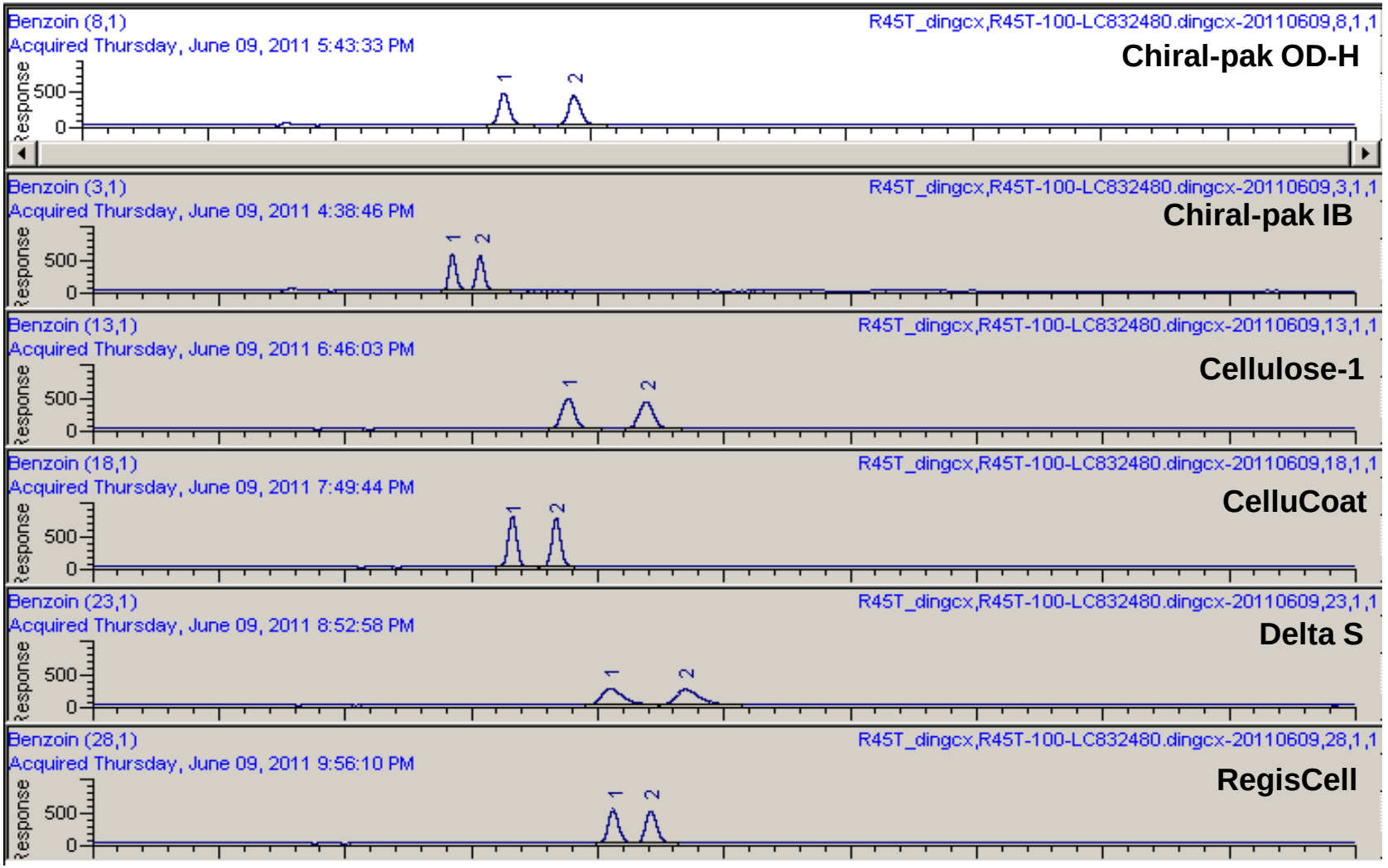
5-Methyl-5-phenylhydantoin



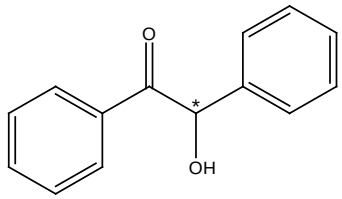
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak OD-H	N/A	N/A	N/A	N/A
Chiralpak IB	N/A	N/A	N/A	N/A
Phenomenex Lux Cellulose-1	N/A	N/A	N/A	N/A
Kromasil 3-CelluCoat	N/A	N/A	N/A	N/A
Macherey-Nagel Nucleocel Delta S	N/A	N/A	N/A	N/A
RegisCell	1.29	4.1	918/841	2.65/3.13

Benzoin, 25% IPA, 75%CO_2



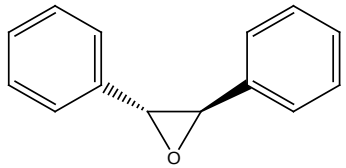


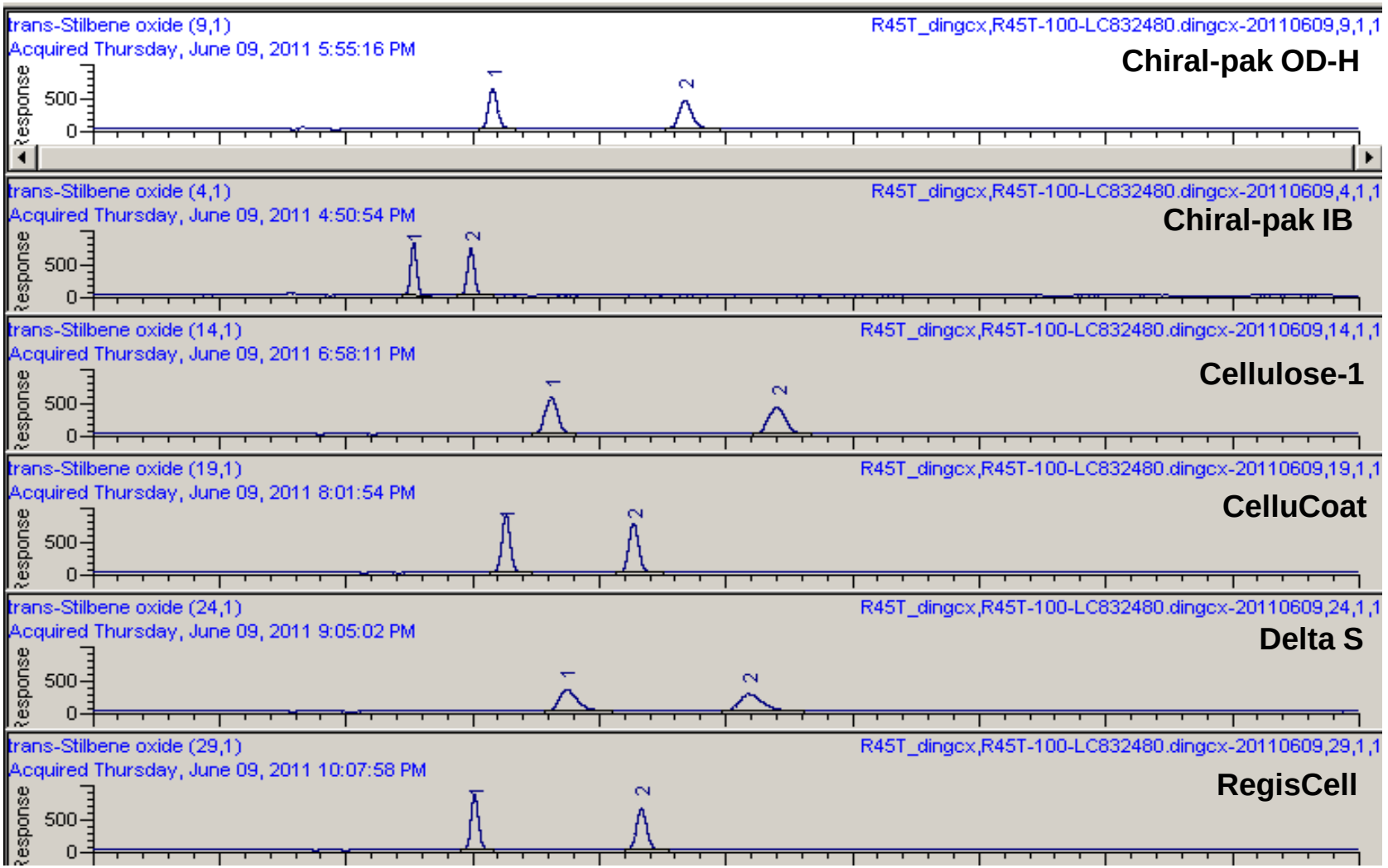
Benzoin



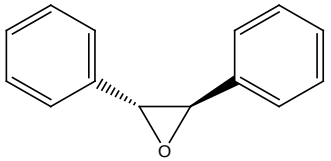
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak OD-H	1.24	3.5	423/384	3.32/3.87
Chiralpak IB	1.12	2.1	541/520	2.85/3.07
Phenomenex Lux Cellulose-1	1.22	3.1	442/399	3.77/4.39
Kromasil 3-CelluCoat	1.15	2.7	745/717	3.33/3.67
Macherey-Nagel Nucleocel Delta S	1.19	1.9	246/221	4.10/4.70
RegisCell	1.09	1.9	503/473	413/442

trans-Stilbene oxide, 25% IPA, 75%CO_2



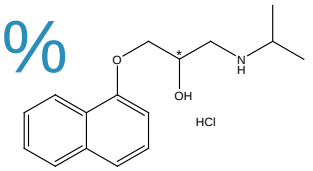


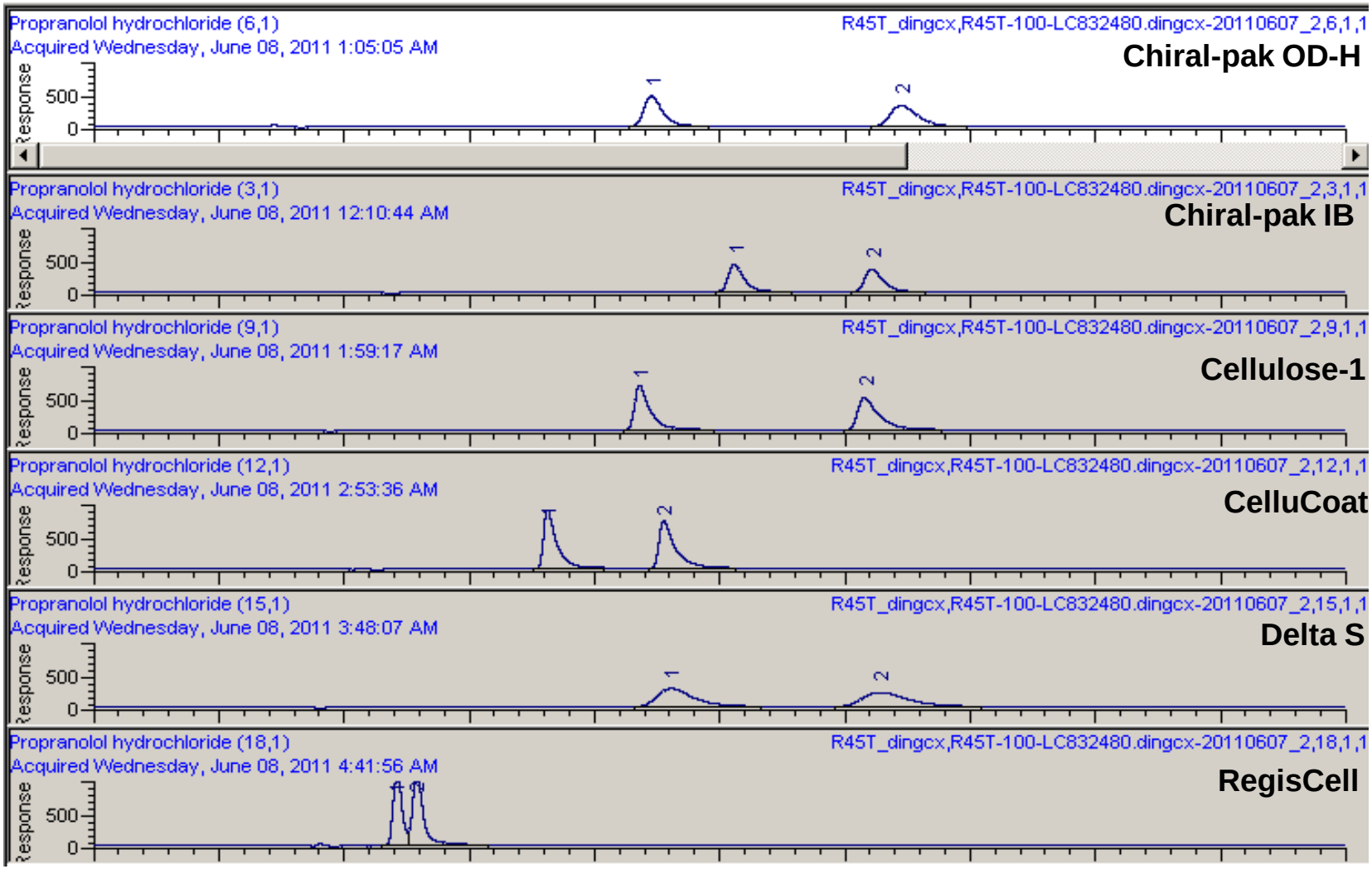
trans-Stilbene oxide



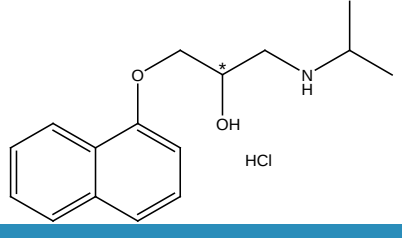
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak OD-H	1.70	9.6	596/416	3.16/4.68
Chiralpak IB	1.29	4.8	774/699	2.54/2.99
Phenomenex Lux Cellulose-1	1.68	8.2	528/386	3.63/5.41
Kromasil 3-CelluCoat	1.44	7.7	868/730	3.27/4.27
Macherey-Nagel Nucleocel Delta S	1.53	4.8	318/250	3.75/5.19
RegisCell	1.65	10.2	811/612	3.02/4.34

Propranolol hydrochloride, 20% MeOH w/ 0.1% DEA, 80%CO_2



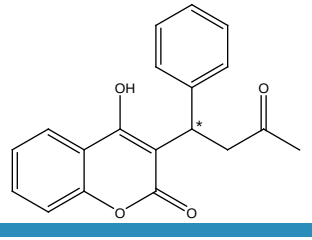


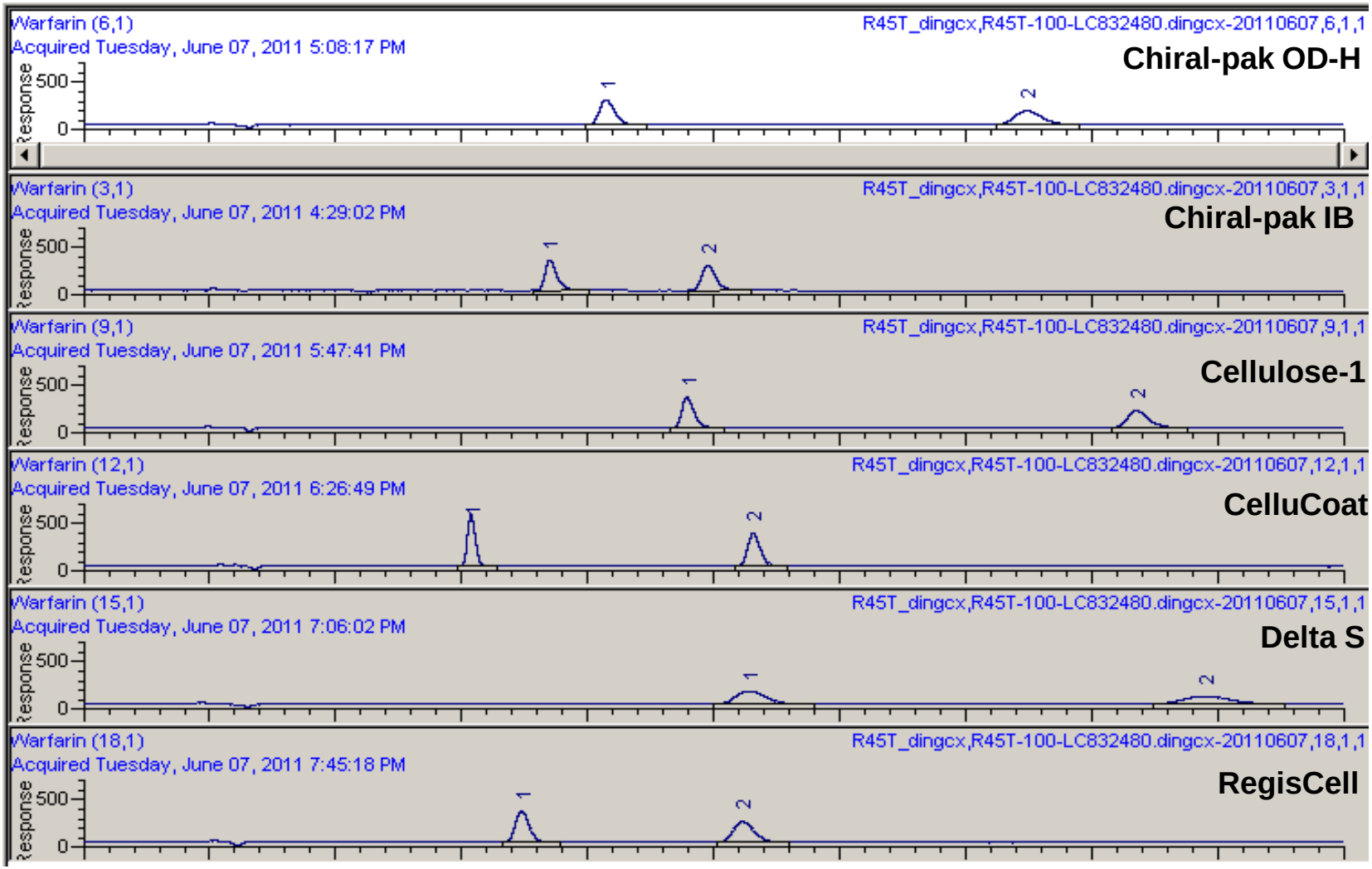
Propranolol hydrochloride



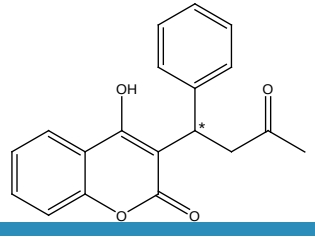
	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak OD-H	1.58	5.9	451/311	4.46/6.45
Chiralpak IB	1.27	4.3	399/331	5.12/6.22
Phenomenex Lux Cellulose-1	1.53	6.9	666/479	4.37/6.16
Kromasil 3-CelluCoat	1.35	4.8	877/704	3.63/4.56
Macherey-Nagel Nucleocel Delta S	1.46	2.8	273/214	4.61/6.29
RegisCell	1.11	1.1	945/943	2.41/2.57

Warfarin, 20% MeOH w/ 0.1% DEA, 80%CO_2





Warfarin



	Enantioselectivity (α)	Resolution (Rs)	Height, mV	RT, min
Chiralpak OD-H	2.06	10.7	262/145	4.15/7.49
Chiralpak IB	1.46	6.8	315/263	3.70/4.96
Phenomenex Lux Cellulose-1	1.94	14.7	320/185	4.79/8.36
Kromasil 3-CelluCoat	2.08	14.7	540/343	3.08/5.32
Macherey-Nagel Nucleocel Delta S	1.84	6.2	139/81	5.28/8.90
RegisCell	1.71	6.9	329/218	3.48/5.23

Conclusion for cellulose-based columns

- Chiralcel OD-H, Lux Cellulose-1 and Kromasil 3-CelluCoat had comparable performance for the chiral separations studied.
- Kromasil 3-CelluCoat gave the highest peak height for each of the compounds studied.
- RegisCell generally showed better performance at conditions with no additive than with additive.

Conclusions

- Columns from different vendors have different performance for some chiral separations.
- No significant advantages were observed for the immobilized phases (IA and IB) with respect to selectivity and resolution.
- No significant advantages were observed for chiral selectivity for the 3 μm particle size columns over the 5 μm columns except for efficiency, which indicated that chiral stationary phases were still the key factors for the chiral separations studied.

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