

Nota applicativa

Evaluation of the General Applicability of SFC for Chiral Separations using a Racemic Compound Library

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Abstract

In this study, we report a systematic investigation on the general applicability of SFC for chiral separations. A total of 176 diverse racemic small molecule compounds were screened; many are commercially available pharmaceutical compounds across several disease areas.

Benefits

By screening a total of 176 diverse, racemic, small molecule compounds, it was demonstrated that SFC is a highly successful chromatographic technique for the chiral separation of small molecule compounds. SFC should be adopted as the technique of choice for chiral analysis and purification across many industries, including pharmaceutical, agrochemical, and nutraceutical.

Introduction

Chiral chromatography, including gas chromatography (GC), capillary electrophoresis (CE), high performance liquid chromatography (HPLC), counter-current chromatography (CCC), and supercritical fluid chromatography (SFC), have been used in chiral resolution.¹ Among these, HPLC and SFC on chiral stationary phases (CSPs) are the most widely utilized chromatographic techniques in drug discovery for obtaining milligrams to multi-grams of pure enantiomers.²

There has been growing awareness of SFC, largely driven by the pharmaceutical industry.¹⁻³ However, they often deal with proprietary compounds for specific disease areas.⁴⁻⁷ For pharmaceutical compounds, factors such as target disease area (e.g. oncology, inflammation, or psychiatry), route of administration (oral or intravenous), and location of target receptor proteins (cell surface, inside cell, or between cells) pose different requirements on the molecules. Consequently, drug candidates for different disease areas may concentrate in different zones of diversity space.

Herein, we report a systematic investigation on the general applicability of SFC for chiral separations. A total of 176 diverse racemic small molecule compounds were screened; many are commercially available pharmaceutical compounds across several disease areas. The experiments were carried out in a parallel manner under selected chromatographic conditions to maximize the success rate while adhering to the principles of speed and simplicity, often required in drug discovery.

Experimental

Compound selection

A total of 176 commercially available small molecule racemates were selected for this study. The structures of the compounds were imported to ChemDBsoft Software (TimTec, Inc., Newark, DE, USA) for diversity analysis. All compounds were dissolved in methanol at 1 to 2 mg/mL.

SFC system components

System:	Method Station X5 SFC System (abbreviated as the "X5 SFC System" hereafter)
Sample manager:	Alias AutoSampler Fluid Delivery Module
Column oven:	Analytical-2-Prep Column Oven (10-port) Automated Back Pressure Regulator
Detection:	Four 2489 Tunable UV/Vis (TUV) Detectors, one 2998 Photodiode Array (PDA) Detector
Data management:	MassLynx Software
Columns:	AD-H, OD-H, OJ-H, AS-H, and IC (4.6 x 250 mm, 5 μ m, Chiral Technologies West Chester, PA, USA)

SFC method conditions

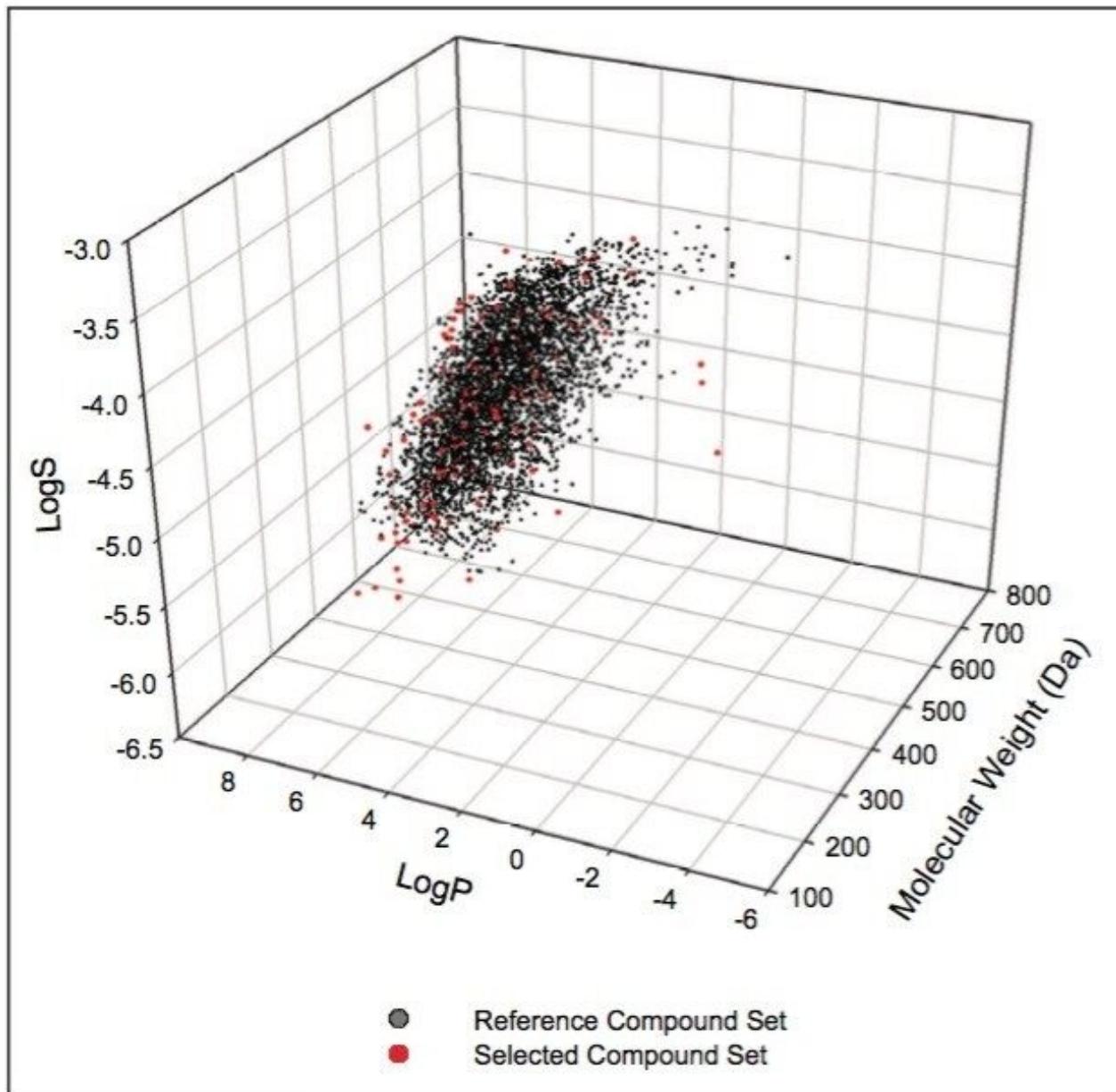
System:	Method Station X5 SFC System (abbreviated as the "X5 SFC System" hereafter)
Sample manager:	Alias AutoSampler Fluid Delivery Module

Column oven:	Analytical-2-Prep Column Oven (10-port) Automated Back Pressure Regulator
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Data management:	MassLynx Software
Columns:	AD-H, OD-H, OJ-H, AS-H, and IC (4.6 x 250 mm, 5 μ m, Chiral Technologies West Chester, PA, USA)

Results and Discussion

Diversity of the compound selection

To ensure that our compound selection represented the types of chemical entities typically encountered in pharmaceutical research while maintaining a manageable sample size, we selected compounds with a wide range of diversity in physical property, structure, and functional group. The compound selection included esters, alcohols, acids, amines, and sulfoxides, to name a few (see Appendix). The structures of these compounds were imported to the ChemDBsoft Software, and the diversity was assessed by the following computed values: Lipinsky score, molecular weight, LogP, LogS, rotational bonds, proton donors, and proton acceptors. The results were then compared to those from TimTec's ApexScreen diverse compound library containing a total of 5,040 compounds.⁸ Figure 1 shows the distributions of our compound selection and the reference ApexScreen diverse library in the chemical space defined by molecular weight, LogP, and LogS. Clearly, albeit being a much smaller sample set, our compound selection covers a similar spatial span as the diverse ApexScreen set. Furthermore, based on the proprietary algorithm of ChemDBsoft for diversity analysis, our compound selection has a numeric diversity score of 0.84; whereas the ApexScreen library has a score of 0.90. Overall, the diversity analysis indicates that our compound selection is sufficiently diverse for the purpose of this evaluation.



Chromatography

When developing a chiral SFC separation, users often start with the screening of multiple columns and multiple mobile phases to determine the optimal chromatographic condition. Currently, there are over 200 commercially available CSPs. However, it has been the experience of many users that derivatized polysaccharides-based CSPs are extremely successful in SFC.^{2, 4-7} The AD-H, OD-H, OJ-H, AS-H, and IC columns were chosen in this study.

In terms of co-solvent selection, methanol and isopropanol were first chosen, as they often induce vastly different selectivity in chiral chromatography. Since derivatized CSPs typically do not need acidic additives

when used in SFC, the two alcohols spiked with 0.2% DEA were also included for basic compounds. An example of the co-solvent effect is shown in Figure 2. With methanol as the co-solvent, there was no separation for promethazine. When isopropanol with 0.2% DEA was used, baseline resolution was achieved on the OJ-H and AD-H columns, and partial separation was achieved on the IC column.

At the drug discovery stage, minimizing the time required to obtain pure enantiomers is of paramount importance.^{2, 9} It should be noted that the primary objective of this study is to gauge the general applicability of SFC for chiral resolution through a "first-pass" or "tier-1" screening,⁶ rather than an exhaustive search for the optimal conditions for each compound. Thus, the experiments were designed to reflect simplicity and speed, which are often required in such analyses at the drug discovery stage. A 6-min generic gradient (5% to 45% in 5 min and 45% to 5% in 1 min) followed by a 2-min re-equilibration (an 8-min injection cycle) was used in this study. However, to run a total of 176 compounds sequentially, with the selected five columns and four co-solvents, it would take a formidable total of 469 hrs ($176 \times 8 \times 5 \times 4 = 28,160$ min) to finish all the experiments. To that end, the X5 SFC System lends itself as a facile solution to expedite the screening process in an automated fashion.¹⁰ In the parallel mode, an injected sample is carried by the mobile phase and divided into five columns. As a result, five columns are screened simultaneously. Two examples are demonstrated in Figure 2. Each injection resulted in five chromatograms. In this study, using the X5 SFC System, all experiments were finished in less than 100 hrs, representing an 80% reduction in the total analysis time.

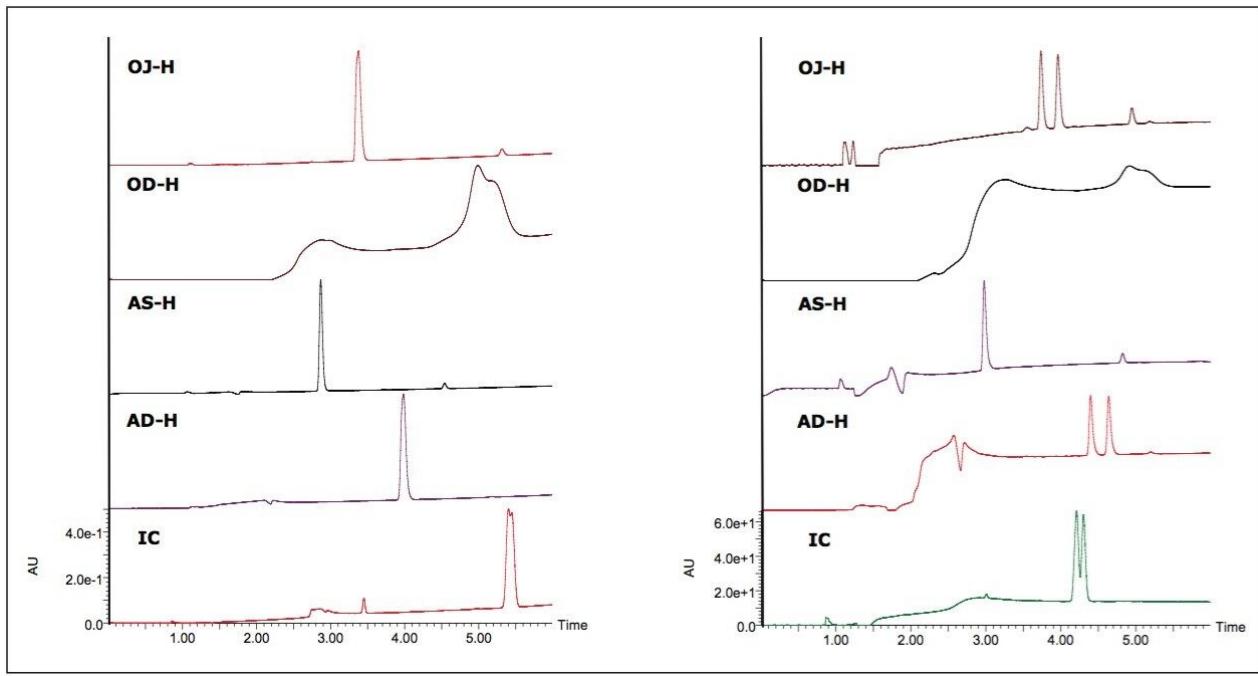


Figure 2. SFC chromatograms of promethazine obtained using a Method Station X5 SFC System with two different co-solvents: left – methanol; and right – isopropanol with 0.2% DEA.

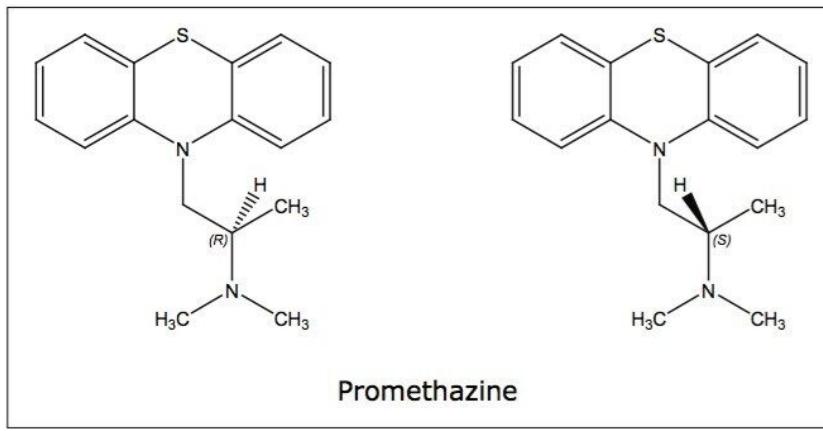


Figure 3. Chemical structure of promethazine.

Evaluation results

All chromatograms were manually inspected. Based on the best chromatogram for each compound, the compounds were categorized as baseline resolved ($Rs > 1.5$), partially resolved ($0.3 < Rs < 1.5$), and not resolved. The results were tabulated in a Microsoft Excel Spreadsheet. In the cases where separations were

observed on multiple columns, the one generating the highest resolution was recorded as the "hit" column.

Among the 176 compounds selected, 146 compounds were baseline resolved, 14 compounds were partially resolved, and 16 compounds were not resolved. The results are summarized in Figure 4. Overall, partial or baseline separations were observed for 91.5% of the compounds; of those, 83.5% of the compounds were baseline resolved. Our observation is similar to the study by Riley *et al.*⁶ In their survey, a total of 120 proprietary compounds were screened using the same five columns used in our study with different co-solvents. An overall 95.6% success rate was reported. It should be noted that this observation was within the boundaries of the selected experimental conditions. In practice, for those partially or not resolved compounds from the "first-pass" screening, additional screening with different columns and/or co-solvents can further improve the success rate.

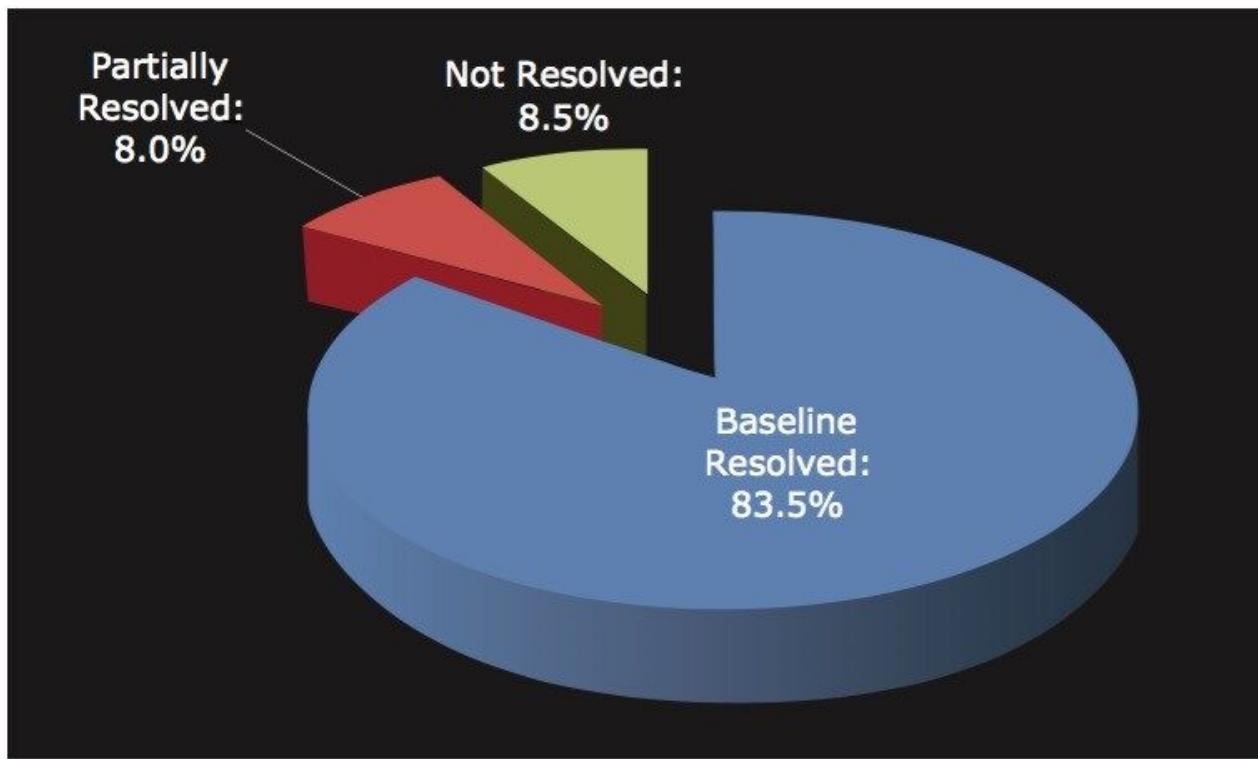


Figure 4. Statistics of the success rate of SFC for separating the selected 176 compounds.

Figure 5 shows the distribution of the column types for the 160 compounds that were baseline or partially resolved. The AD-H column yielded the highest number of "hits", while the IC and AS-H columns shared the second place. The AS-H and OD-H columns were the distant fourth and fifth, respectively. These results support the perception that no single column is overwhelmingly "universal." Even with the AD-H column, the success rate was only 37%. These observations also agree with other reports⁴⁻⁷ that AD-H is the most

successful derivatized polysaccharides-based CSP in terms of general applicability. However, it is noteworthy that the order of columns in terms of success rate varies from study to study. Barnhart *et al.*⁴ reported that for 40 commercial compounds, the order was AD-H > OD-H > AS-H > OJ-H > P-CAP; however, for 100 proprietary Amgen compounds under the same experimental conditions, that order became OD-H > AD-H > AS-H > OJ-H > P-CAP. In the study reported by Riley *et al.*,⁶ the order was AD-H > OD-H > OJ-H > IC-H > AS-H for 120 proprietary Pfizer compounds. There are several reasons that could contribute to the observed discrepancy. First, the order of columns is highly compounddependent, as demonstrated by Barnhart *et al.*⁴ Two different orders were generated for two different sets of compounds, while other conditions remained same. As a result, caution should be exercised to ensure sample diversity when a general conclusion is drawn. Secondly, the makeup of the selected columns could also change the "hit" distribution. An ideal column selection for screening should include a minimal number of columns with complementary selectivity to maximize the success rate. And finally, the selection of co-solvents also contributes to the "hit" column distribution. This is evidenced by the comparison between our results and the one reported by Riley *et al.*⁶ From a chromatography perspective, the main difference lies in the selection of co-solvents. Yet, the OD-H column ranked second in their study and ranked last in our study.

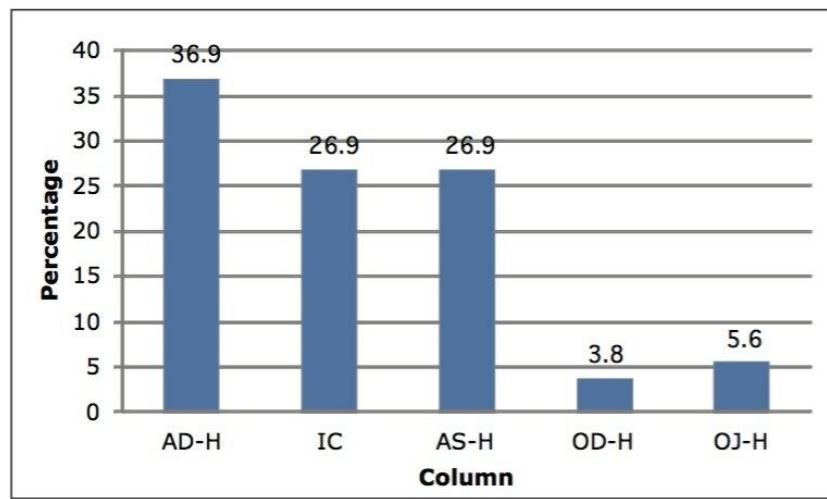


Figure 5. Distribution of column type for baseline and partially resolved compounds.

Conclusion

In this study, we have screened a total of 176 commercially available racemic small molecule compounds, including many pharmaceutical compounds, to evaluate the general applicability of SFC for chiral separations. The selected compounds cover a sufficiently wide range of diversity in structure, functional group, and physical property. Under selected conditions, separations for 91.5% of the selected compounds were observed; among those, 83.5% of the compounds were baseline resolved. In addition, the use of a parallel Method Station X5 SFC System expedited the screening process and improved the throughput by five-fold. The results indisputably indicate that SFC is a highly successful chromatographic technique for the chiral separation of small molecule compounds. This, along with the environmental sustainability and cost-effectiveness, should stimulate a wider adoption of SFC for chiral analysis and purification across many industries, including pharmaceutical, agrochemical, and nutraceutical.

References

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Appendix: Summary of the evaluation results

Compounds with No Separation (a total of 16)		Compounds with Partial Separation (a total of 14)	
Number	Compound	Number	Compound
17	Chloroquine	1	Acebutolol
21	2-chloroadenosine	2	Allethrin
22	DL-3,4-MDMA	4	Aspartame
23	N-Imidazol-N'-carbonic acid-3,5-dinitroanilide	19	Cytidine
29	Metolachlor	20	N-CBZ DL valine
45	Morphine sulfate	36	Isolutrol
53	p-methoxyamphetamine	43	Mequitazine
64	Ritalinic acid	46	1-(1-naphthyl) ethylamine
93	Tetramethrin	49	Novobiocin
104	Codeine	54	(±)- α -Pinene
117	4-(Trifluoromethyl)-mandelic acid	110	1-phenyl-1-propanol
150	Vanilmandelic acid	111	1-phenyl-2-propanol
151	3,5-difluoro mandelic acid	113	2-Chloromandelic acid
153	Baclofen	115	2-Phenylpropionic acid
160	Ephedrine hydrochloride		
164	Norphenylephrine HCl		

Compounds with Baseline Separation (a total of 146)

Number	Compound	Column	Number	Compound	Column
3	Alprenolol	AD-H	92	Diniconazole	AS-H
5	Atenolol	OD-H	94	Tebuconazole	AD-H
6	Atropine	IC	95	Hexaconazol	AD-H
7	Bendroflumethiazide	AD-H	96	Oxprenolol	IC
8	Benzoin	AD-H	97	Cyclothiazide	IC
9	Binol	AS-H	98	Praziquantel	AS-H
10	Bucetin	AD-H	99	Promethazine	AD-H
11	Bupivacaine	AD-H	100	Sotalol	AD-H
12	Bupropion HCl	AD-H	101	Mandelic acid	IC
13	(L)-chlorpheniramine	IC	102	Hexobarbital	IC
14	Carboxamine	AD-H	103	Methadone	OJ-H
15	Carprofen	AS-H	105	Cetirizine	AS-H
16	Chlorcyclizine	AS-H	106	Devrinol	IC
18	Citalopram	IC	107	1,2,3,4-tetrahydro-1-naphthol	AS-H
24	Ethosuximide	AS-H	108	1-Indanol	AS-H
25	Fenoprofen	AD-H	109	1-Phenyl-1,2-ethanediol	AD-H
26	Fenvalerate	AD-H	112	2-(3-chlorophenoxy) propionic acid	OJ-H
27	Flavanone	IC	114	2-phenylbutyric acid	AD-H
28	Flurbiprofen	AD-H	116	(R, S)-4-benzyl-2-oxazolidinone	AS-H
30	Guaiacol glyceryl ether	AD-H	118	Abscisic acid	AS-H
31	Guanosine	AS-H	119	Acenaphthanol	IC
32	Hydroxyzine	AS-H	120	α -phenylethanol	OJ-H
33	(\pm)-Isoproterenol	AS-H	121	Aminoglutethimide	AS-H
34	Ibuprofen	AD-H	122	Butabarbital	AD-H
35	Indapamide	AS-H	123	Coumachlor	IC
37	Ketoprofen	IC	124	Cyclopentobarbital	AD-H

38	Labetalol	AS-H	125	Cyclopentylphenylacetic acid	AD-H
39	Lorazepam	AS-H	126	Dichlorprop	AD-H
40	(\pm)-3,4-methylenedioxy-amphetamine	IC	127	Diclofop Methyl	IC
41	(\pm)-metoprolol	OD-H	128	Diperodon	AS-H
42	Mephenesin	AD-H	129	DL-5-(4-hydroxyphenyl)-5-phenylhydantoin	AS-H
44	Methylphenidate HCl	AD-H	130	DL-tropic acid	AD-H
47	1-(2-Naphthyl)-1,2-ethanediol	IC	131	Ethyl mandelate	AD-H
48	Nadolol	AD-H	132	Etodolac	AS-H
50	Omeprazole	AS-H	133	γ -phenyl-gamma-butyrolactone	IC
51	Orphenadrine	AD-H	134	Hydrobenzoin	IC
52	Oxazepam	AS-H	135	Indoprofen	AS-H
55	1-Phenylcyclohexane-cis-1,2-diol	AS-H	136	Mephobarbital	OJ-H
56	Permethrin	OJ-H	137	Metalaxyl	AD-H
57	Pindolol	AD-H	138	Nimodipine	IC
58	Proglumide	IC	139	Orphenadrine	AD-H
59	Propafenone	AD-H	140	Resmethrin	OJ-H
60	Propranolol	OD-H	141	Secobarbital	AD-H
61	Sulconazole	AS-H	142	Styrene oxide	AD-H
62	Sulfinpyrazone	AS-H	143	Sulindac	IC
63	Sulpiride	AS-H	144	Tetrahydronbenzo(a)-pyrene-7-ol	IC
65	4',5,7-Trihydroxyflavanone	AS-H	145	Thalidomide	AD-H
66	DL-Tartaric acid	OD-H	146	Tofisopam	IC
67	Terbutaline	AS-H	147	Triadimefon	AD-H
68	Tetramisole	IC	148	Troger's Base	AD-H
69	Timolol	IC	149	Vinyl phenyl sulfoxide	AS-H
70	Trans-stilbene oxide	AD-H	152	1,1'-binaphthol 2,2'-diamine	AD-H
71	\pm -Verapamil	AD-H	154	2-amino-1-phenylethanol	AD-H
72	Warfarin	OD-H	155	(\pm)-2-amino-3-phenyl-1-propanol	IC
73	DL-Malic acid	AS-H	156	Benzoin methyl ester	AS-H
74	Ketoconazole	AS-H	157	(R, S)-benzyl mandelate	AS-H
75	Disopyramide	AS-H	158	Fenoterol HBr	AD-H
76	Salbutamol	AS-H	159	Fipronil	IC
77	Clenbuterol	AD-H	161	Methyl mandelate	AS-H
78	Miconazole	AS-H	162	(\pm)-N-N'-bis(α -methylbenzyl)sulfamide	AD-H
79	Tropicamide	AD-H	163	(\pm)-N-N'-dimethyl-1-(1-naphthyl)ethylamine	AD-H
80	Flecainide	AD-H	165	Octapamine	IC
81	Nicardipine	IC	166	Phenoxybenzamine	OJ-H
82	Econazole	AS-H	167	Phenylalanine-methyl ester	IC
83	Triadimenol	AS-H	168	Proline	AD-H
84	Fluoxetine	AD-H	169	Tolperisone	IC
85	Mianserin	AD-H	170	Trichlormethiazide	IC
86	4-chlorophenylalanine methyl ester	IC	171	Trihexyphenidyl	IC
87	Tramadol HCl	AD-H	172	Trimebutine	OJ-H
88	Thioridazine HCl	AD-H	173	Trimipramine	IC
89	Lansoprazole	AS-H	174	Trolox	AD-H
90	Pantoprazole	IC	175	β -Methyl-phenethylamine	AD-H
91	Oxfendazole	IC	176	5-methyl-5-phenylhydantoin	AS-H

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