

Capillary Electrophoresis

METHODS DEVELOPMENT STRATEGY FOR ENANTIOMER ANALYSIS USING THE PIACE™ MDQ CHIRAL SYSTEM

Jeff Chapman, Harry Whatley, Fu-Tai A. Chen
Beckman Coulter, Inc.

Introduction

Whether one's approach to drug discovery involves natural products screening or the synthesis of innovative new compounds, the challenges presented to the analytical chemist are compounded when a product contains one or more chiral centers. Yet these optically active compounds make very good drug candidates.

Enantiomers are stereoisomers that display chirality, that is having one or more asymmetric carbon centers, allowing them to exist as non-superimposable mirror images of one another. These isomers are difficult to analyze as they are both physically and chemically identical and differ only in the way they bend plane-polarized light and in their behavior in a chiral environment. The first step toward separating enantiomers is to create diastereomers. Diastereomers may be created through chemical derivatization with a "chiral" reagent, or they may be formed transiently through interactions with chiral selectors. The latter is usually the most desirable as it is the easiest to employ.

The construction of a chiral environment in capillary electrophoresis (CE) is achieved by simply filling the capillary with a buffer containing a chiral additive. Although many chiral selectors have been used, the most comprehensive and effective separation strategies have been achieved with sulfated cyclodextrins. In this case, the degree and the site of sulfation appear to significantly impact the separation of enantiomers.⁽¹⁾

Highly Sulfated Cyclodextrins

In this application note, we describe a methods development strategy using a family of highly sulfated cyclodextrins (HSCDs) developed at Beckman Coulter—these include HS- α , HS- β , and HS- γ cyclodextrins. Each of the HSCD reagents consists of a controlled distribution of the product, designed as part of a strategy for simplified chiral methods development. Perhaps the most remarkable characteristic of these HSCDs is the ability to provide separation for enantiomers of different structures without the need for variation in separation conditions. A single generic method approach is presented here.

Our experience with HSCDs shows that the vast majority of successful separations may be achieved at low pH.⁽¹⁻⁴⁾ Under these conditions of suppressed electroosmotic flow (EOF), the negatively charged cyclodextrins have strong electrophoretic mobility toward the positive electrode (anode). If the enantiomers interact with these HSCDs, they will be swept toward the anode regardless of charge state. Neutral compounds will interact with the hydrophobic cavity of the HSCDs, while basic compounds will be strong cations at low pH—interacting with the hydrophobic cavity and ionically with the negatively charged sulfates. At pH 2.5, acidic compounds will be primarily protonated, behaving as neutral species. Zwitterionic analytes will be positively charged and behave in a fashion similar to basic compounds. Figure 1 illustrates the analysis of

these four classes of enantiomers using a single analytical condition. Typically we recommend that your methods development strategy include three primary runs—screening each of the cyclodextrin types for the one which achieves the best resolution.

Although trends are developing that should allow future prediction of the best starting selector, we recommend implementing a strategy which utilizes the α , β and γ selectors independently to determine which one will provide the best resolution of your analyte. At low pH, the EOF in an untreated fused-silica capillary is minimal. In this case, the electrophoretic mobility of an analyte (μ) in the presence of HSCD can be represented by a weighted sum of the mobility of free analyte μ_F and that in the complexed state, μ_C , as shown in equation (1).

$$\mu = \mu_F + K_1(\text{HSCD})\mu_C / [1 + K_1(\text{HSCD})] \quad (1)$$

In this equation, the (HSCD) term is the concentration of free HSCD after interaction with the analyte. If this number is small, the mobility of the complex would be affected significantly by a change in analyte concentration. For this reason, we recommend a starting concentration of the HSCD at 5% (25 mM) as this should be one to two orders of magnitude greater (typically 0.1 to 1 mM) than the concentration of the chiral species being separated. The excess amount of chiral selector over the chiral species makes the [free HSCD] virtually independent of variations in analyte concentration. And, as a greater population of analyte will be in the complexed state, a net higher mobility of analyte will result.

The mobility of two enantiomers (racemates), 1 and 2, can be compared according to equation (2).

$$\mu_1 - \mu_2 = \{ \mu_{F1} + K_1(\text{HSCD}) \mu_{C1} / [1 + K_1(\text{HSCD})] \} - \{ \mu_{F2} + K_2(\text{HSCD}) \mu_{C2} / [1 + K_2(\text{HSCD})] \} \quad (2)$$

Since HSCDs are highly negatively charged with substantially greater molecular weight than that of the analytes being separated, the mobility of the complex can be approximated as $\mu_{C1} = \mu_{C2}$ and nearly equal to μ_{HSCD} . Neutral enantiomers should satisfy the condition of $\mu_{F1} = \mu_{F2}$. Thus, equation (2) can be modified as:

$$\mu_1 - \mu_2 = (\mu_{C1} - \mu_{F1}) (K_1 - K_2) (\text{HSCD}) / [1 + K_1(\text{HSCD})] [1 + K_2(\text{HSCD})] \quad (3)$$

For a neutral species, $\mu_{F1} = \mu_{F2} = 0$, and for basic compounds, $\mu_{C1} > \mu_{F1}$.

Thus,

$$\mu_1 - \mu_2 = \mu_{\text{HSCD}} (K_1 - K_2) (\text{HSCD}) / [1 + K_1(\text{HSCD})] [1 + K_2(\text{HSCD})] \quad (4)$$

In principle, the mobility difference, $\Delta\mu$, is intrinsically related to K_1 and K_2 and, when [HSCD] is substantially large, a small variation of racemate concentration would not affect the total free (HSCD); thus, the $\Delta\mu$ (resolution term) will remain essentially constant. This is what we see in practice, when maintaining a high concentration of chiral selector. In such cases the resolution term and migration time remains relatively constant even with changing analyte concentration.

HSCD Consistency

The consistency of the chiral selector supply is also of great importance. Historically, cyclodextrins used for chiral analysis have been notorious for lot-to-lot variability. The reason was due primarily to the manufacturing intent of these reagents, typically produced in large scale for applications other than chiral analysis. Conversely, the Beckman Coulter HSCDs are produced for the purpose of enantiomer analysis. These reagents are manufactured using a controlled sulfation reaction and formulated to produce consistent lots. All HSCDs are developed using strict quality control procedures which test the reagents' consistency in functional assays. Table 1 illustrates the consistency of the migration time of PTS, glutethimide, and pseudoephedrine across different lots of these reagents. The solid synthetic lot GS6276-67 is the gold standard. Within the same lot, the run-to-run reproducibility was well below 1% RSD while the comparison of each solution lot with the gold standard was below 4%. Assessment of analyte resolution is specified as part of our formulation criteria.

Elemental analysis of the α , β , and γ HSCDs show the average sulfate content to be 11, 12, and 13 sulfates per mole respectively. The ^{13}C NMR is consistent with the structural assignment of sulfation at C-6 primary hydroxyl groups and the C-2 secondary hydroxyls (>70%), while the C-3 hydroxyls remain intact. This appears to be a key factor in determining the resolution of the enantiomers.

Figure 2 illustrates a proposed mechanism by which differential enantiomer interaction may occur.

Although racemic mixtures may be baseline resolved at a calculated resolution greater than 1.0, detection of low-level enantiomers can be easily masked when the concentration of the eutomer (major enantiomer) is increased to the level necessary to detect the lower-concentration enantiomer. However, as resolution values of 5 or greater are commonly obtained using the HSCD approach, low-level enantiomer quantitation below 0.1% levels can be readily achieved. Figure 3 illustrates the detection of (-) phenylpropanolamine enantiomer at 0.1%. The good resolution afforded by this assay makes this formerly difficult task routine.

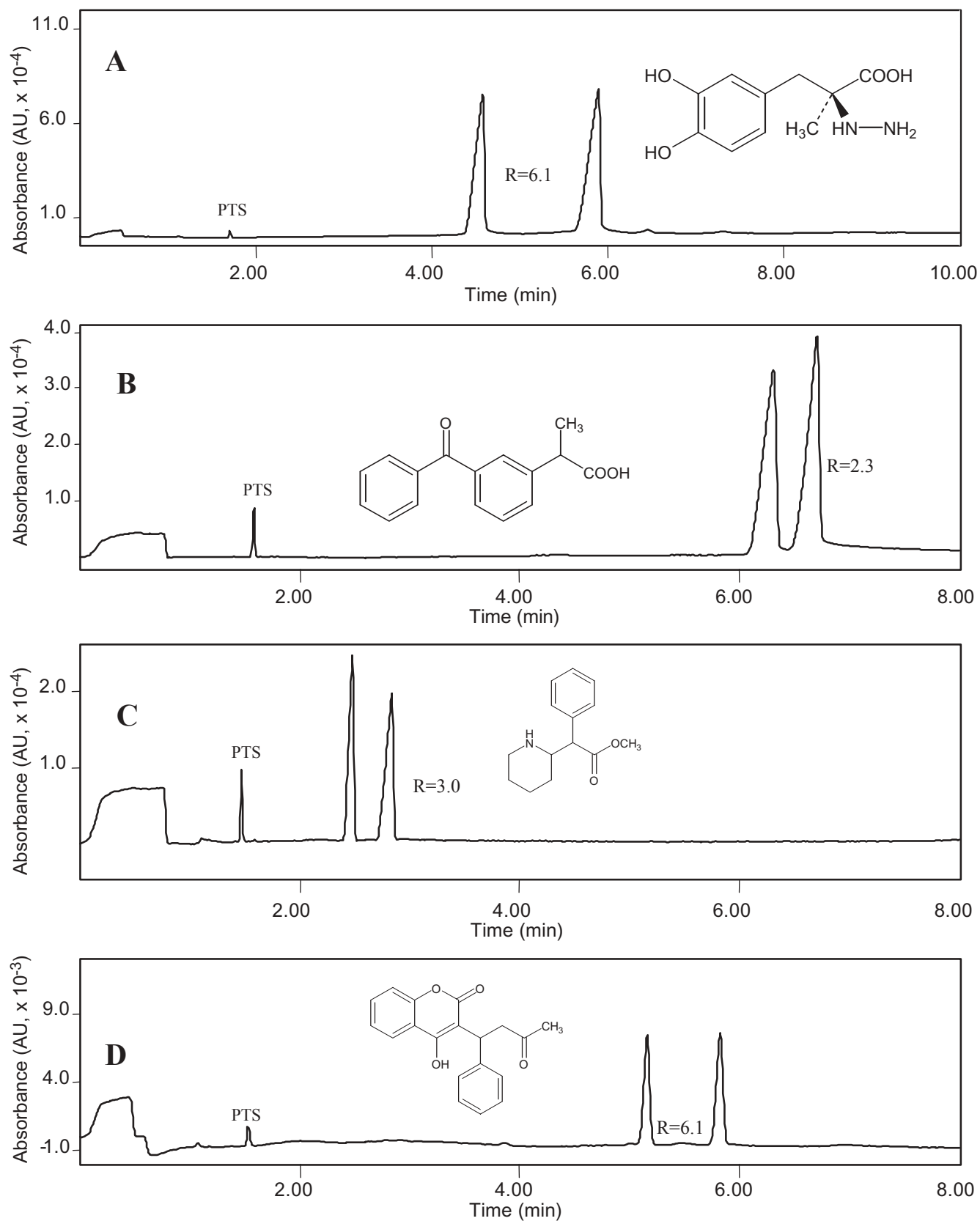


Figure 1. Electropherograms of zwitterionic, acidic, basic, and neutral chiral compounds using 5% β -HSCD in 25 mM phosphate buffer pH 2.5. Panel A: Carbidopa (Zwitterionic drug), B: Ketoprofen (Acidic drug), C: Ritalin (Basic drug) D: Warfarin (Neutral drug). Conditions: Untreated fused-silica capillary 50 μm \times 30 cm (20 cm to detector). Capillary temperature 22°C (recirculating liquid); field strength 500 Volts/cm; detection: PDA 200 nm; sample introduction: pressure, 1.2 p.s.i*seconds; current generated: 156 μamps .

Table 1

<i>Solution Lot</i>	<i>Solid Lot</i>	<i>Pseudoephedrine</i>			<i>Glutethimide</i>		
D711B902	GS6276-67	PTS tm	tm1	tm2	PTS tm	tm1	tm2
Gold Std 5-run mean		1.46	2.45	2.74	1.46	2.44	3.33
Gold Std 5-run RSD		0.65%	0.42%	0.37%	0.27%	0.11%	0.05%
D710B913	D709A101						
5-run mean		1.50	2.37	2.67	1.50	2.44	3.26
5-run RSD		0.29%	0.33%	0.35%	0.22%	0.13%	0.15%
Dev from Gold Std		2.20%	3.40%	2.65%	2.42%	0.07%	2.02%
D710B914	D710A101						
5-run mean		1.51	2.36	2.67	1.52	2.44	3.31
5-run RSD		0.18%	0.19%	0.17%	0.42%	0.20%	0.31%
Dev from Gold Std		3.28%	4.05%	2.36%	3.51%	0.25%	0.70%

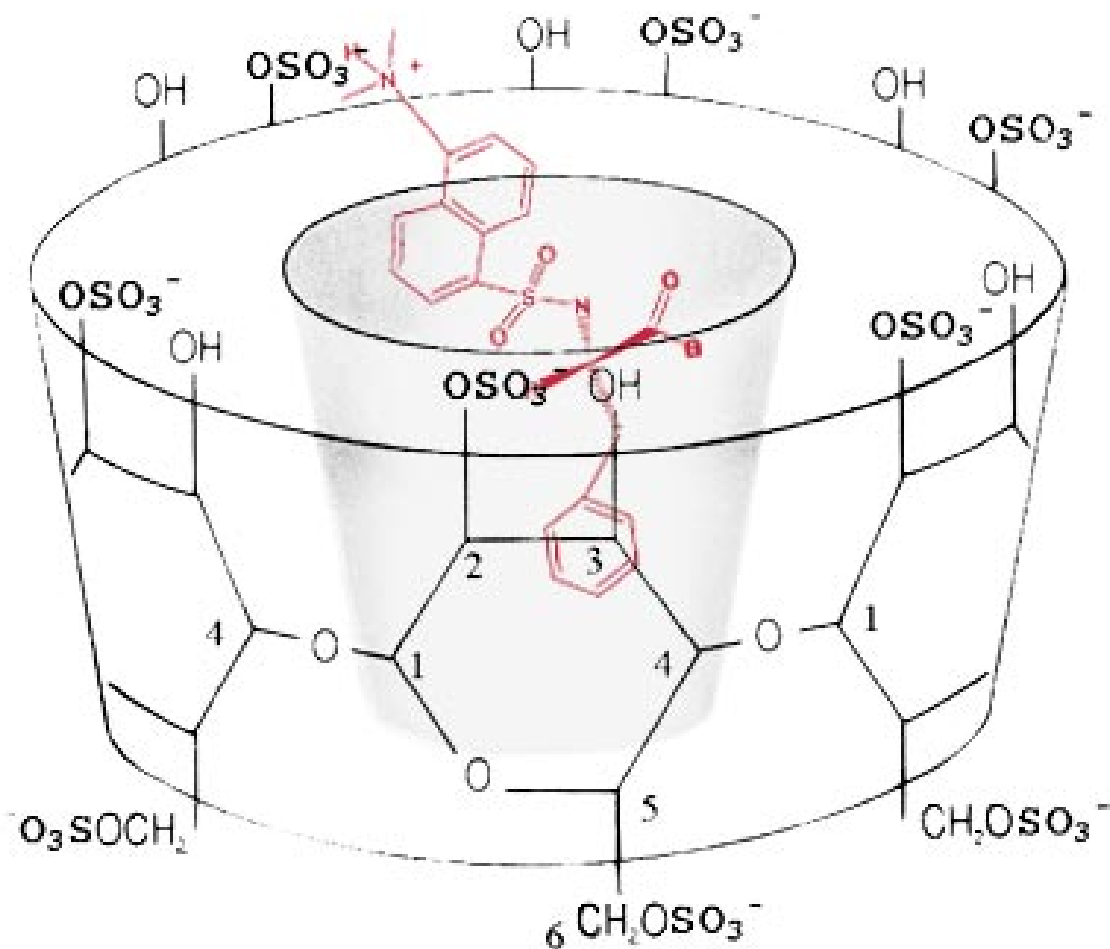


Figure 2. Proposed mechanism of interaction between DNS-phenylalanine and HS- γ -CD. A key interaction point for chiral recognition appears to be at the C3 primary hydroxyl. Sulfation at this point interfered with enantiomeric discrimination (data not shown).

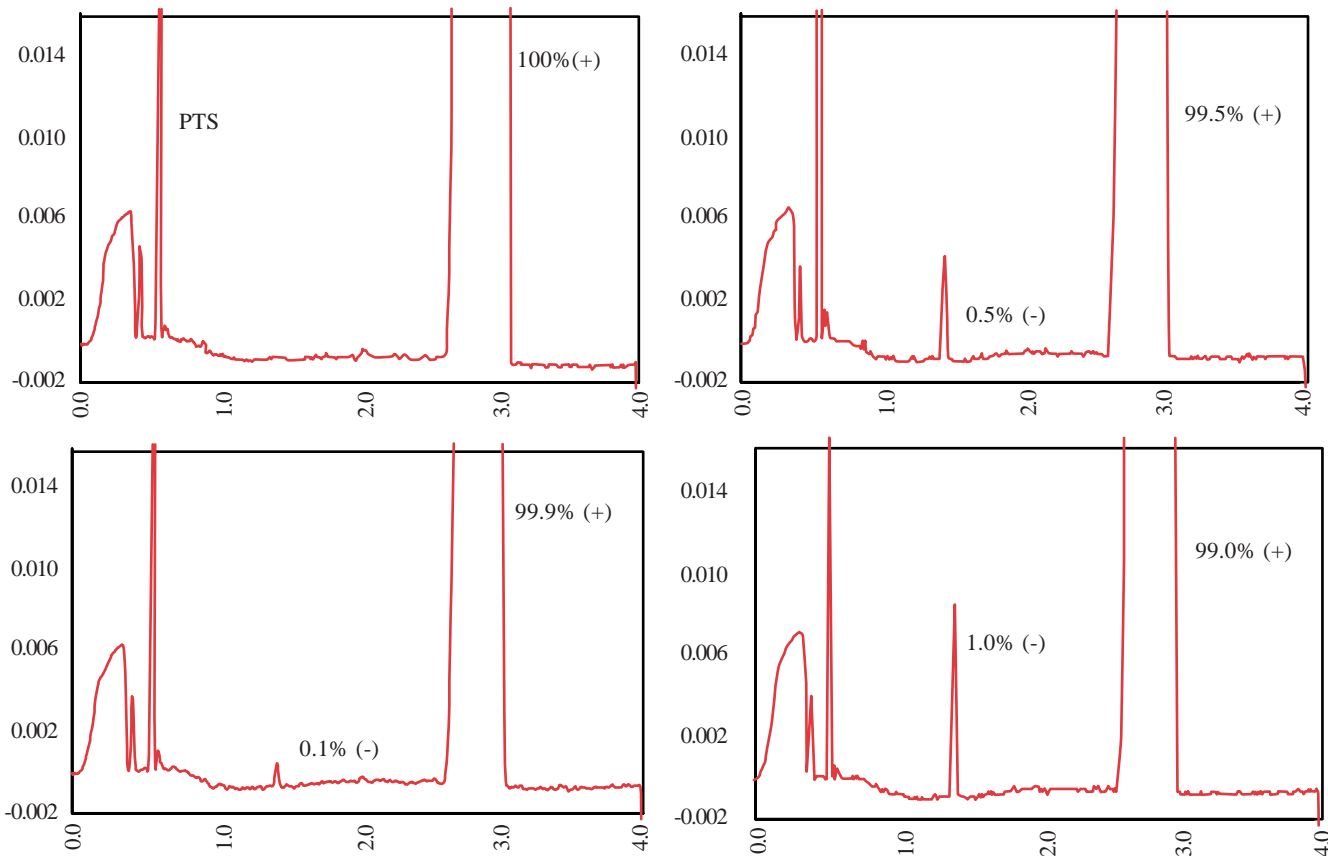


Figure 3. Electropherogram of a dilution series of contaminating phenylpropanolamine enantiomer with the (-) form readily detected even at 0.1%. Conditions—Buffer: 5% γ -HSCD in 25 mM phosphate, pH 2.5. Capillary: untreated fused-silica capillary, 50 μm x 30 cm (20 cm to detector). Capillary temperature: 22°C (recirculating liquid). Field strength: (-)500 Volts/cm (anode at outlet). Detection: PDA, 200 nm. Sample introduction: pressure, 1.2 p.s.i*seconds; current generated: 152 μAmps .

Instrumentation

These rapid and efficient chiral separations require the advanced liquid cooling of the P/ACE™ MDQ. Due to the conductivity of the HSCDs, high currents are generated, requiring effective capillary thermoregulation. A variety of sampling formats, including a 96-well plate, allows this system to be compatible with many forms of laboratory automation.

Evaluating the similarity of an analyte's spectra provides valuable information to the analyst developing methods for enantiomer analysis. With the P/ACE MDQ chiral system, photodiode array (PDA) detection is employed to achieve this task. As enantiomers are physiochemically identical, they should have matching spectra, allowing you to discriminate enantiomer pairs from contaminating analytes. Figure 4 illustrates the assistance of PDA in identifying the enantiomers from other components present in the electropherogram. In this example, glutethimide enantiomers are distinguished from the added reference marker 1,3,6,8-Pyrenetetrakisulfonate.

Although most drug candidates are UV-absorbing, there are cases where the chiral compound of interest does not have significant UV absorbance. In these examples, one can employ a pre-separation derivatization to render either a UV-absorbing or fluorescing product. An example is shown in Figure 5 with the analysis of homoproline. Shown is the separation of dansylated homoproline enantiomers—both as a racemic mixture and a single enantiomer. In this example, less than 0.1% of the contaminating enantiomer was detected.

The linearity of detection is also an important consideration. Figure 6 illustrates the P/ACE MDQ PDA detector response linearity for the phenylpropanolamine enantiomers described above. This plot describes the % impurity as a function of the ratio of the velocity-corrected peak area of the contaminant/total. A good linear response is generated, allowing an accurate determination of enantiomer impurity even at levels as low as 0.1%.

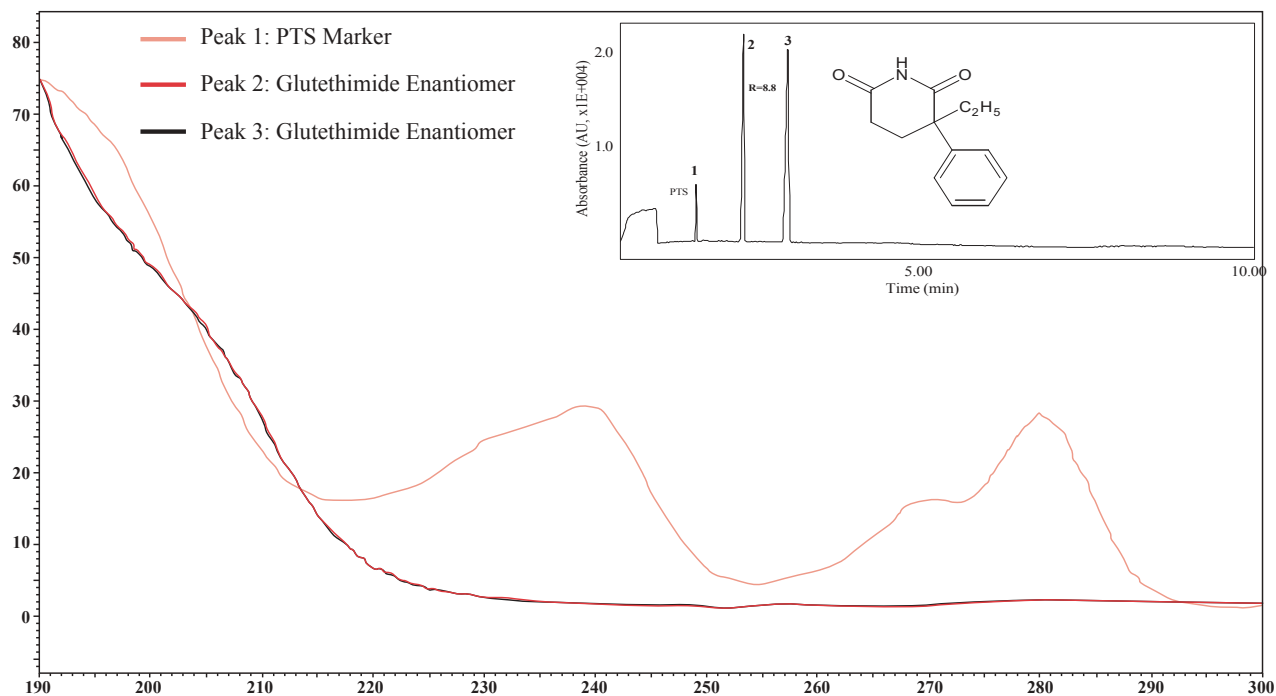


Figure 4. Utility of photodiode array detection in assisting the identification of enantiomer pairs from other contaminating analytes. In this example, glutethimide enantiomers are readily distinguished from the reference marker (PTS).

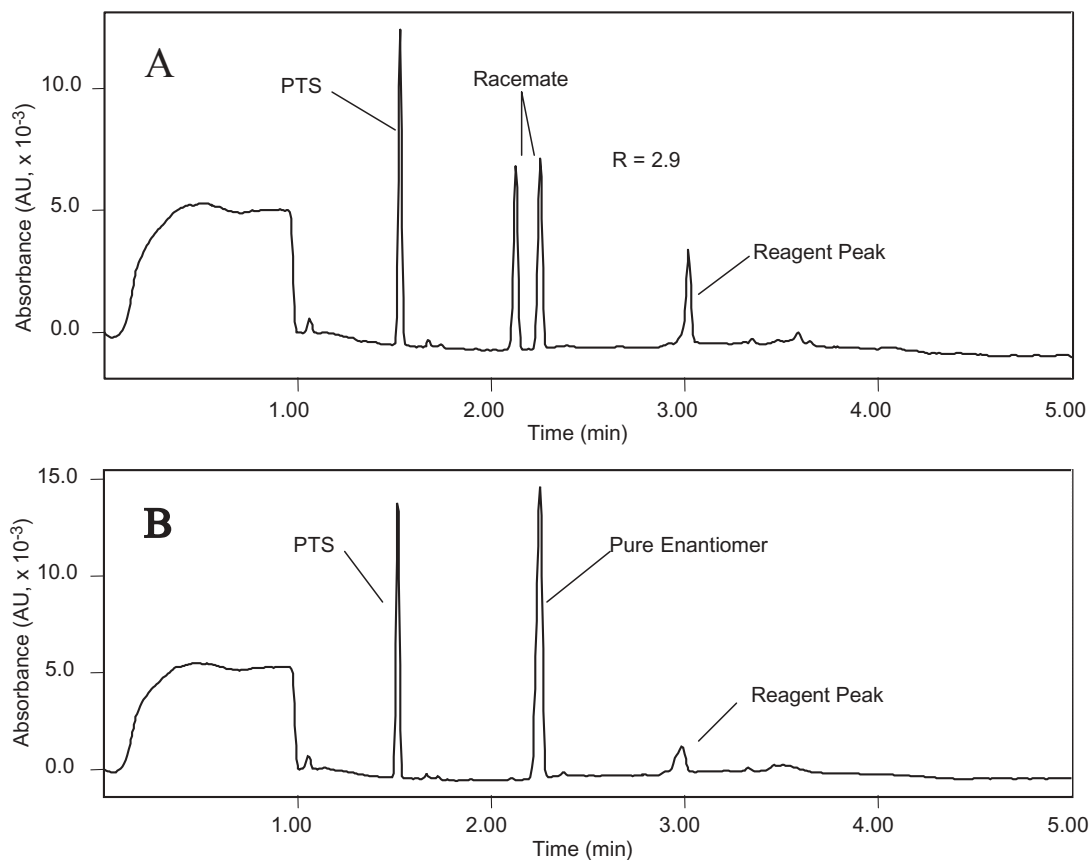


Figure 5. Electropherogram of dansylated derivatives of homoproline. Panel A: Racemic mixture of homoproline. Panel B: L-homoproline enantiomer. Conditions—Buffer: 5% γ -HSCD in 25 mM phosphate, pH 2.5. Capillary: untreated fused-silica capillary, 50 μ m x 30 cm (20 cm to detector). Capillary temperature: 22°C (recirculating liquid). Field strength: (-)500 Volts/cm (anode at outlet). Detection: PDA, 200 nm, Sample introduction: pressure, 1.2 p.s.i*seconds. Current generated: 155 μ Amps.

Capillary Selection and Conditioning

A 50- μm -I.D. bare fused-silica capillary x 20 cm (to detector; 30 cm total length) is recommended for this strategy. This capillary dimension allows you to operate under high field strengths, while still maximizing mass load. Of course, once a separation has been achieved, you may wish to optimize resolution/speed by reversing the polarity and introducing the sample from the short side of the capillary. In this case, a 10-cm capillary can be used to generate a very rapid

analysis of enantiomers. Figure 7 illustrates an example of the rapid assay of dansylated phenylalanine enantiomers. A resolution greater than 3 is generated within only a minute of separation time.

As the separation occurs at low pH, we do not recommend NaOH rinses between runs. Rinsing with run buffer prior to each analysis is usually sufficient, although we do recommend rinsing at least once a day with a polyethylene oxide solution. This acts to coat the capillary surface, allowing a much

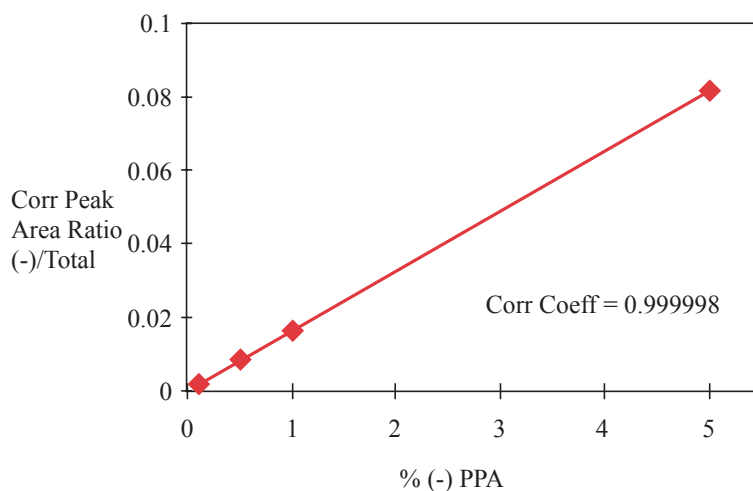


Figure 6. Plot of the ratio of the impurity velocity-corrected area/total velocity corrected peak area versus the percent of contaminating enantiomer (impurity) for phenylpropanolamine. A linear response from 0.1% to 5% is readily attained, allowing effective quantitation of the lower-level enantiomer to levels as low as 0.1%.

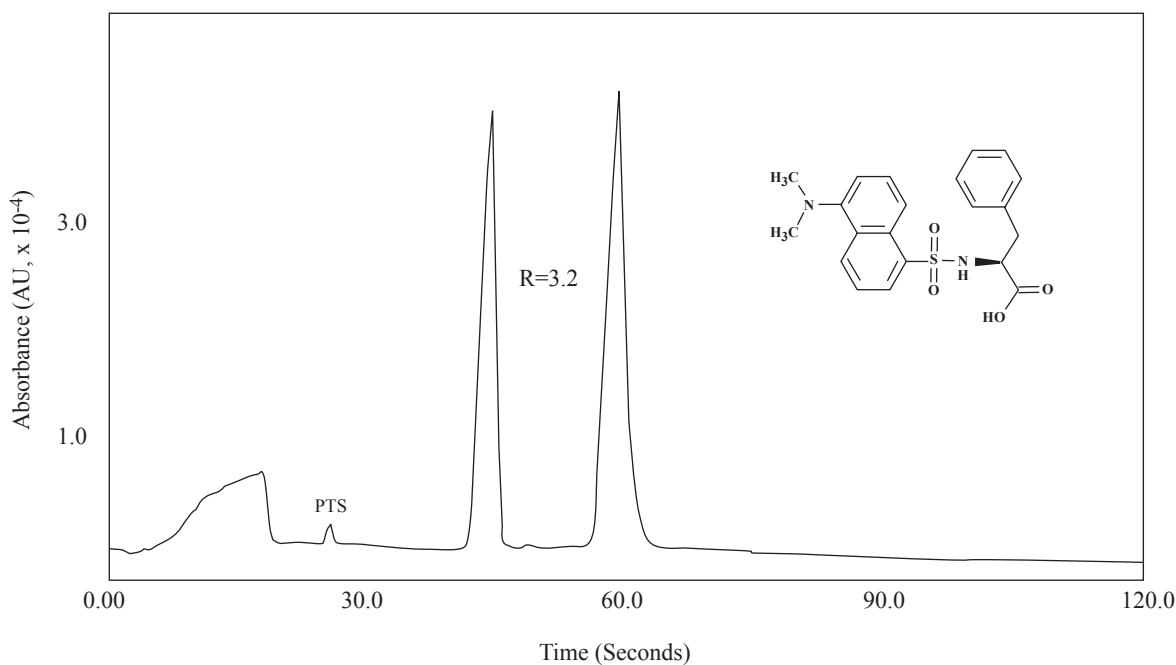


Figure 7. Electropherogram of dansylated-phenylalanine enantiomers separated using a short capillary (10 cm—sample introduction from the outlet side). Conditions—Buffer: 5% γ -HSCD in 25 mM phosphate, pH 2.5. Capillary: untreated fused-silica capillary, 50 μm x 30 cm (10 cm to detector). Capillary temperature: 22°C (recirculating liquid). Field strength: (+)500 Volts/cm (cathode at outlet). Detection: PDA, 200 nm. Sample introduction: pressure, 1.2 p.s.i*seconds, sample introduced from outlet side. Current generated: 157 μAmps .

faster equilibration. This polymeric conditioning solution is available from Beckman Coulter (P/N 713333).

This solution is made up of the following:

- 25 mM lithium acetate
- 0.4% PEO (MW 300,000)
- 10% ethylene glycol
- pH 4.75

Run Parameters

In order to simplify comparative analysis, we recommend that you standardize your run conditions during the initial screening runs. Once the best cyclodextrin type and concentration are determined, you can begin the process of optimization. The starting conditions that we recommend are as follows:

- Capillary temperature: 22°C
- Field strength: 500 Volts/cm
- Detection: PDA, UV 200 nm;
Scanning, 190-320 nm
- Sample introduction: 1.2 psi*sec (4 seconds at 0.3 psi)

Water dips to prevent sample carryover/contamination may be inserted after both sample introduction and the end of the run.

Another important variable is the total programmed run time. As the resolution of enantiomer pairs can be quite high, one can easily mistake a failed separation attempt with simply not allowing enough separation time to resolve the two enan-

tiomers. In Figure 8, we illustrate the analysis of amphetamine enantiomers where such an oversight could easily be made. Notice the elapsed run time from the first enantiomer to the second. If the run were aborted too soon, one might have made the assumption no separation was achieved. A good experiment to consider is to run your sample with and without the chiral selector. If complete resolution is achieved, the peak heights will be approximately half that of the control where no selectors were present. Alternatively, you can use the capillary reversal method using a short capillary (10 cm) to reduce the total run time required.

Methods Optimization

Once the primary three runs (evaluating the α , β , and γ HSCDs) have been performed, an optimization process may be implemented. The decision tree shown in Figure 9 summarizes the typical steps one may take in the optimization process.

Choose the HSCD which gave the best resolution. You may wish to vary the concentration between 2 and 10% to evaluate any enhancement of resolution; however, our recommendation is to use 5%, when possible, as this maintains the cyclodextrin concentration high enough to prevent separation changes that result from changes in analyte concentration. Capillary temperature, capillary length, and field strength may all be used to fine-tune the resultant

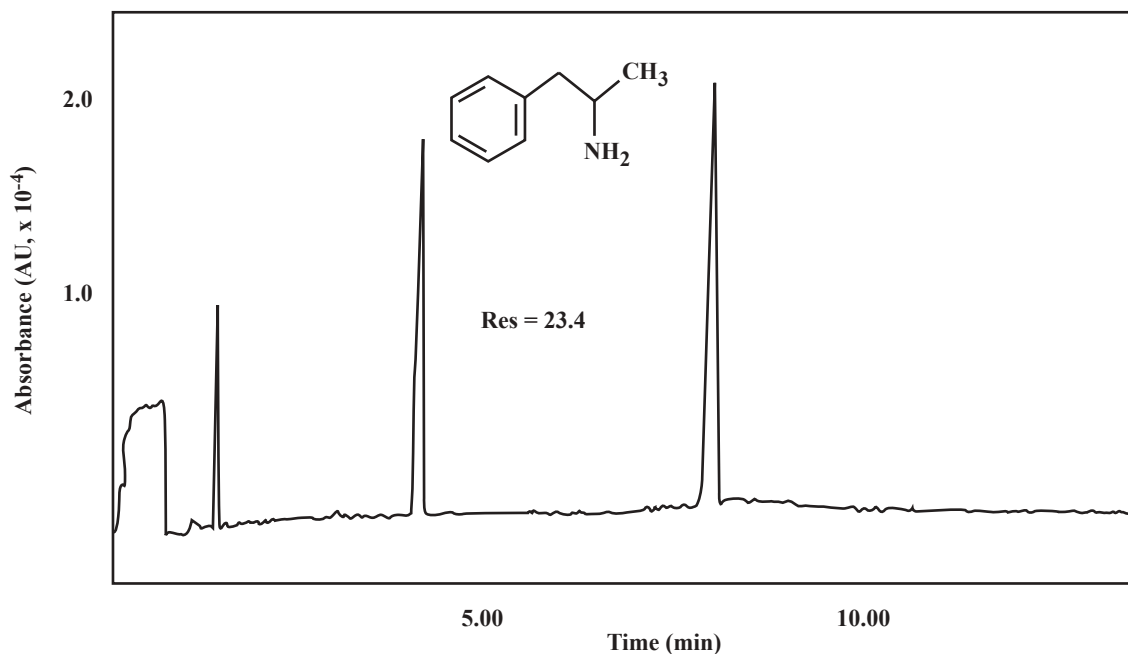


Figure 8. Electropherogram of amphetamine enantiomers demonstrating the high-resolution achieved with highly sulfated cyclodextrins. Conditions—Buffer: 5% γ -HSCD in 25 mM phosphate, pH 2.5. Capillary: untreated fused-silica capillary, 50 μ m x 30 cm (20 cm to detector). Capillary temperature: 22°C (recirculating liquid). Field strength: (-)500 Volts/cm (anode at outlet). Detection: PDA, 200 nm. Sample introduction: pressure, 1.2 p.s.i*seconds. Current generated: 156 μ Amps.

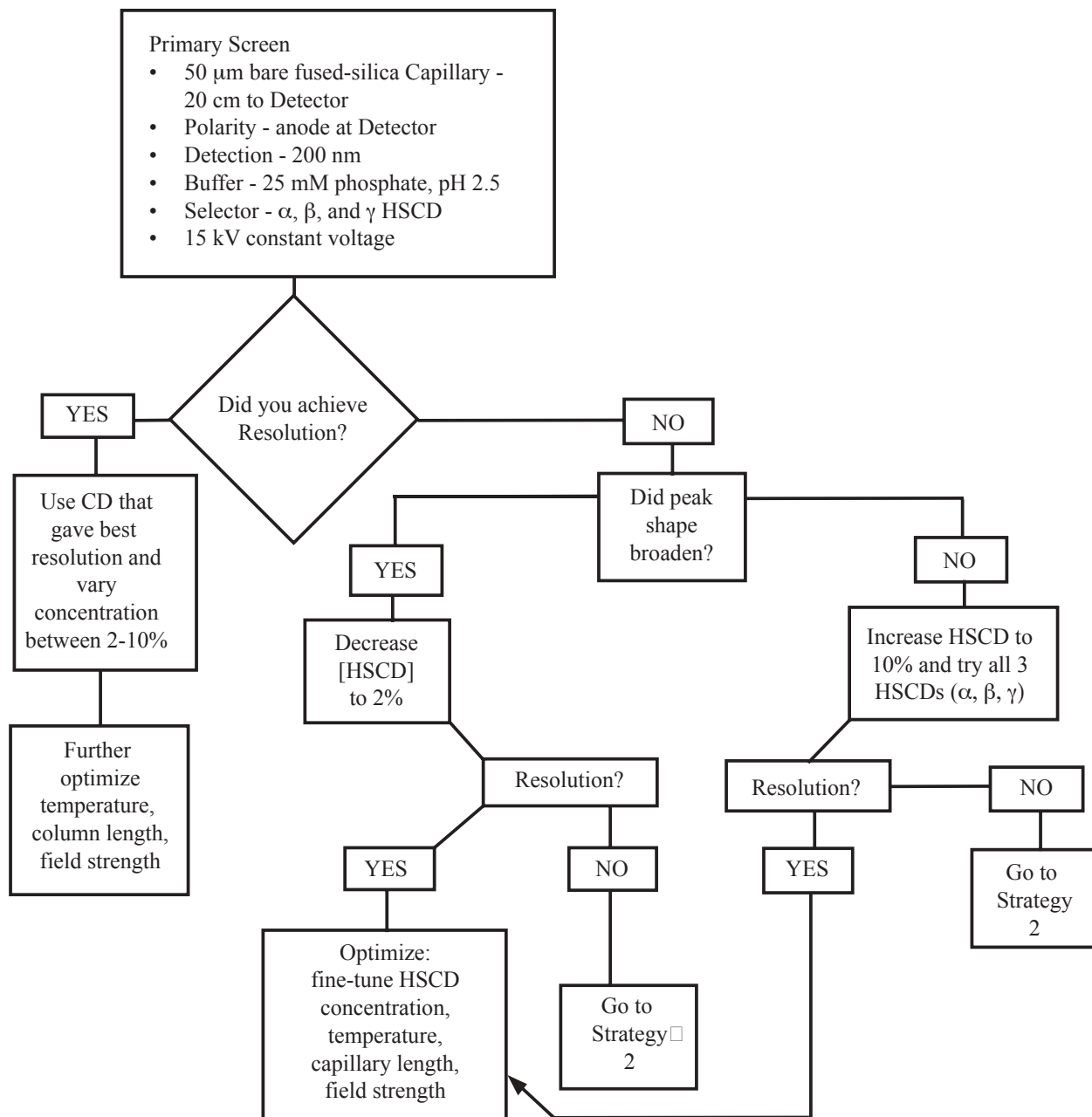


Figure 9. Decision tree for method optimization process.

method. Typically, the field strength should be optimized last, once you have decided upon the optimum capillary length and temperature. As the resolution achieved with HSCDs is usually quite high, the trade-off of using a 10 cm capillary to reduce the analysis time is a very valuable parameter. In many cases, 10 cm will yield excellent resolution while generating total analysis times less than 1 or 2 minutes.

If resolution of the enantiomers was not achieved with any of the three cyclodextrins, you should focus your attention first to the peak shape.

Did any of the runs illustrate a broadening of peak shape? If the answer is yes, there is a good

chance your HSCD concentration is too high, forcing a stronger interaction than desired. In this case, try lowering the concentration. An HSCD dilution series of 1 to 5% can rapidly provide you with the data for a standard curve to estimate the best concentration of selector to choose. If there was no change in peak shape, you may wish to increase the HSCD concentration in a series from 5 to 10%. Of course, if you are operating as an overnight screen, you may choose to automate this process by using the P/ACE™ MDQ buffer array. Figure 10 illustrates an example of how you may wish to set up the buffer array.

Buffer Arrays

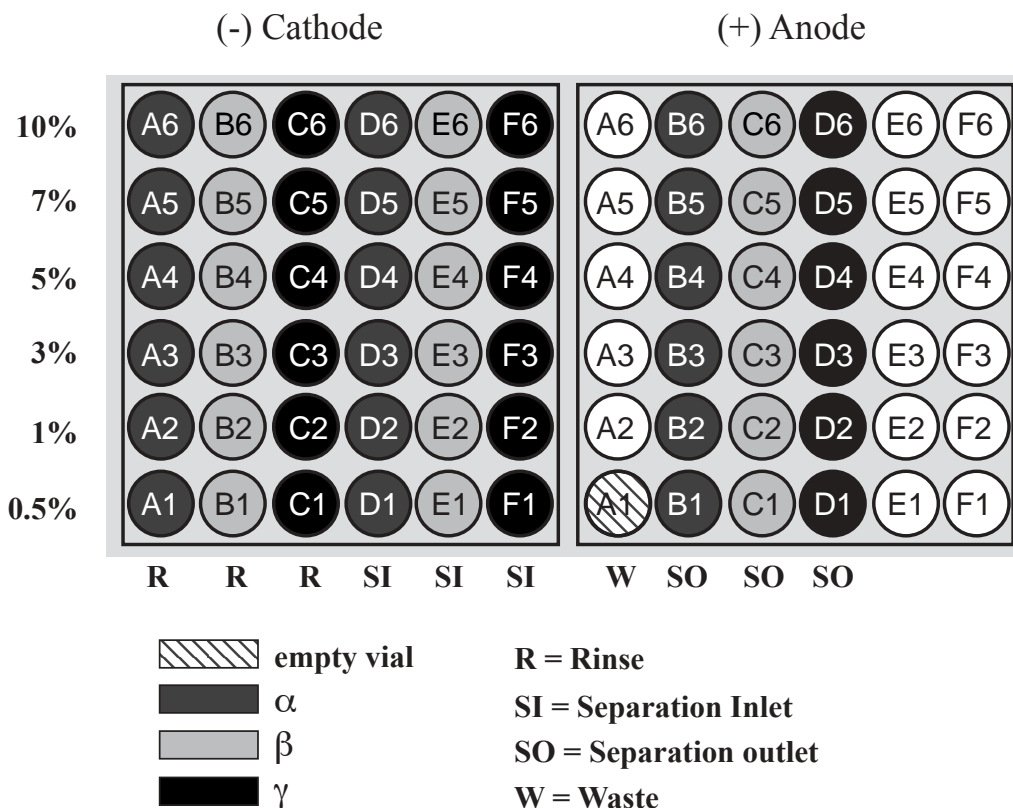


Figure 10. Proposed setup of P/ACE™ MDQ buffer array for the automation of the optimization process. A single method setup with buffer increments can be used to scout through this tray.

Results

The results achieved using this method development strategy have been quite impressive. Using only the primary screen of α , β , and γ HSCD and the standard method described above, 156/160 drugs tested have been resolved ($R > 1.0$) with no optimization employed. The compounds tested included:

- 56/58 Neutral drugs
- 92/92 Basic drugs
- 8/10 Acidic drugs

Examples of many of these drugs can be found in Appendix II of this application bulletin or on the internet at www.beckmancoulter.com/chiral38

In summary, we have presented a powerful chiral analysis strategy using a combination of the P/ACE™ MDQ and a family of highly sulfated cyclodextrins. The result is a sound enantiomer analysis strategy which utilizes a single method approach. The benefits are rapid and intuitive methods development at reduced cost, yielding faster and more reliable results.

References

1. Chen, F-T. A., Evangelista, R. A., Chin, J. *Chem. Soc.* 46, 847 (1998)
2. Stalcup, A., Gahm, K. H. *Anal. Chem.* 68, 1360 (1996)
3. Verleysen, K., Sabah, S., Scriba, G., Chen, A., Sandra, P. *J. Chromatogr.* 824, 91-96 (1998)
4. Verleysen, K., Van den Bosch, T., Sandra, P. *Electrophoresis* 20, 2650 (1999)

Appendix I

P/ACE™ MDQ Chiral System

149833 North America

285402 International

A CE-based analytical system configured optimally for developing methods for the separation of enantiomers. The system includes a P/ACE MDQ configured with a photo diode array detector, UV source optics, ambient sample storage module and P/ACE MDQ Software configured on an IBM personal computer. Installation Qualification, Operation Qualification 1 (OQ1) and documentation to aid in software validation is also included.



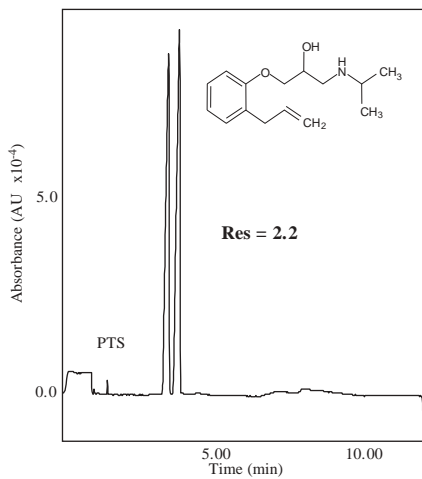
Starter chemistries include: The Highly Sulfated Cyclodextrins Chiral Methods Development Pack (strategy #1), Neutral CD Chiral Methods Development Kit (strategy #2), and Chiral Methods Development Guide.



Appendix II

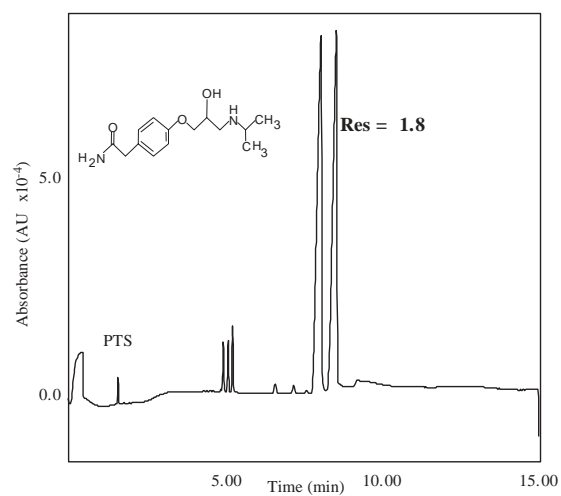
Alprenolol

HS- α -CD



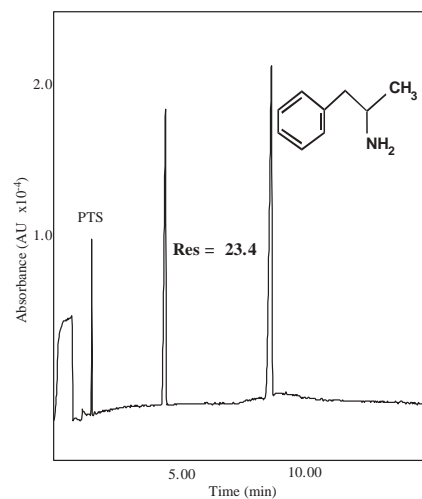
Atenolol

HS- γ -CD



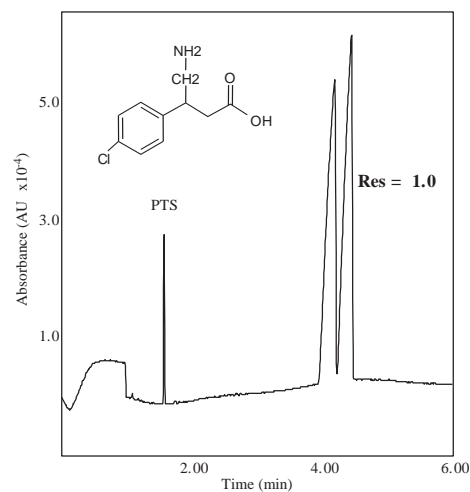
Amphetamine

HS- γ -CD



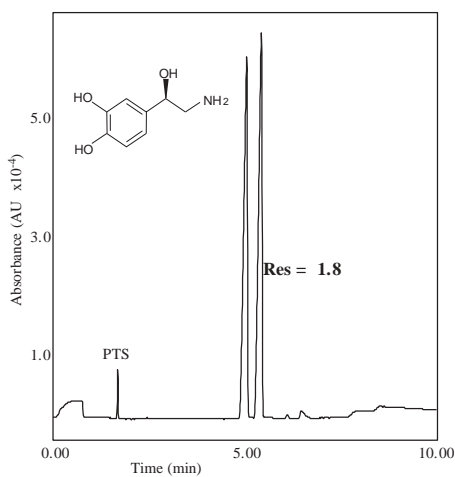
Baclofen

HS- α -CD



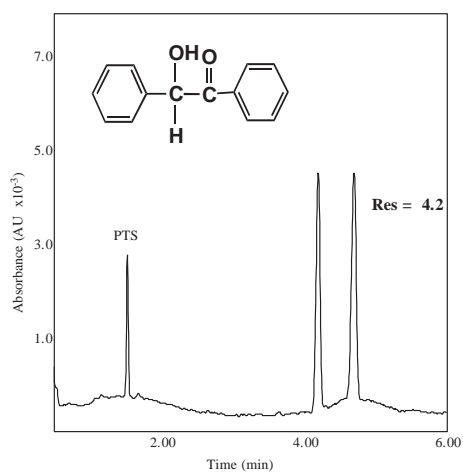
Arterenol (norepinephrine)

HS- γ -CD



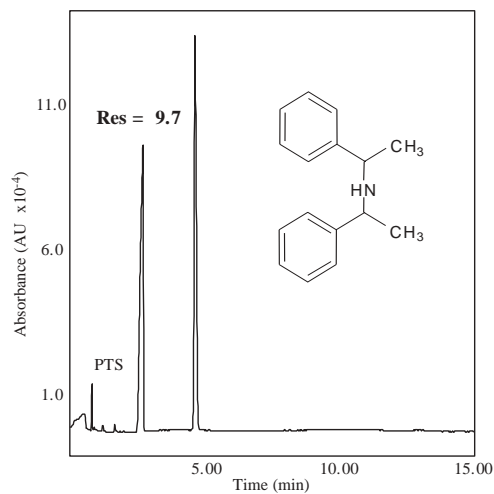
Benzoin

HS- β -CD



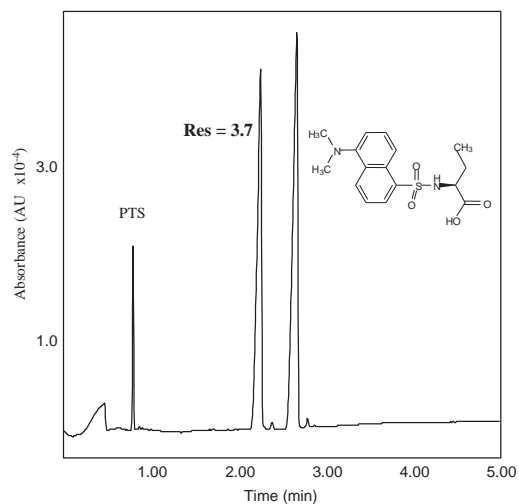
Bis-(1-Phenylethyl) amine (10 cm)

HS- γ -CD



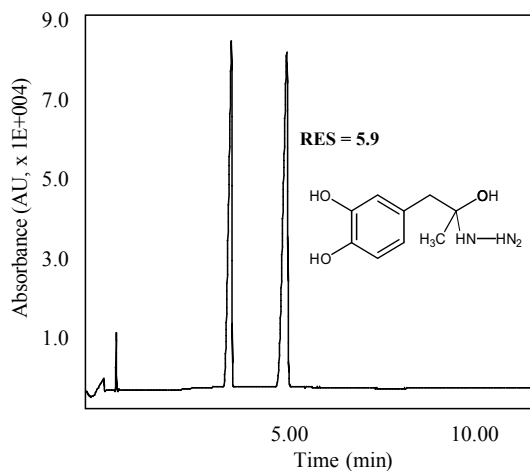
DNS- α -aminobutyric acid (10 cm)

HS- γ -CD



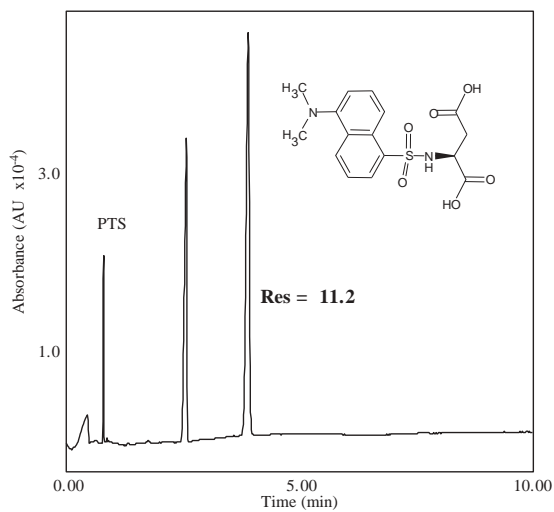
Carbidopa

HS- β -CD



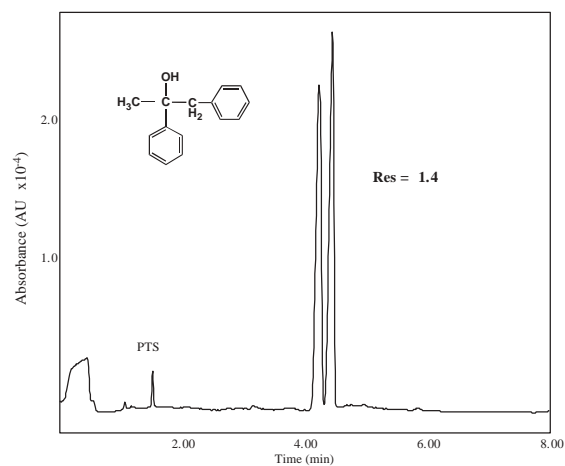
DNS-aspartic acid (10 cm)

HS- γ -CD



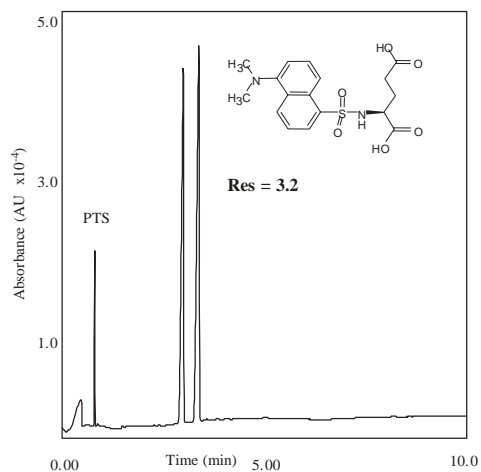
1,2-Diphenyl-2-propanol (DPP)

HS- β -CD



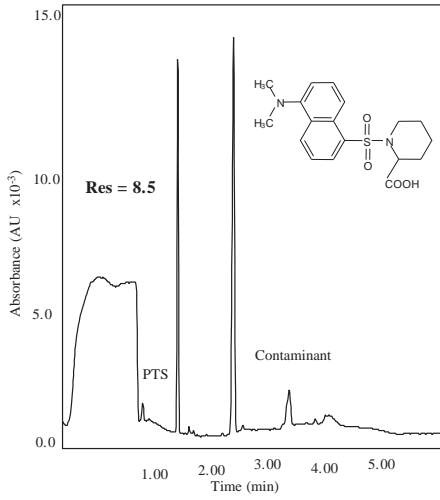
DNS-glutamic acid (10 cm)

HS- γ -CD



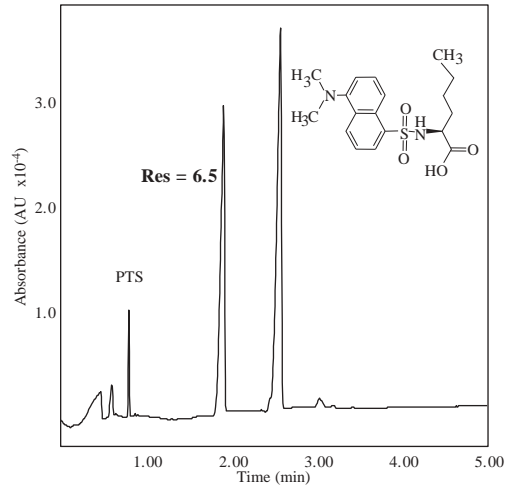
DNS-homoproline (10 cm)

HS- γ -CD



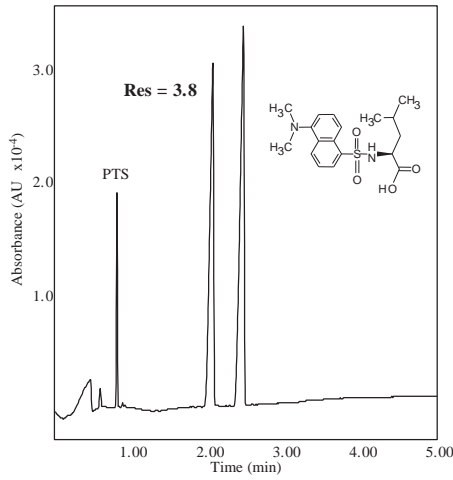
DNS-norleucine (10 cm)

HS- γ -CD



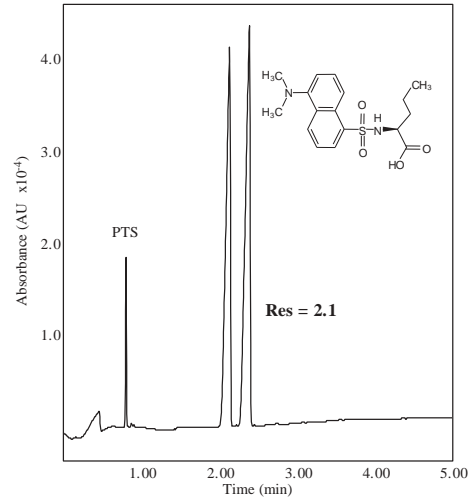
DNS-leucine (10 cm)

HS- γ -CD



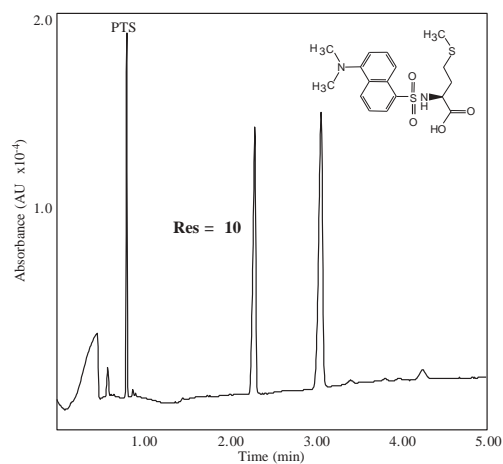
DNS-norvaline (10 cm)

HS- γ -CD



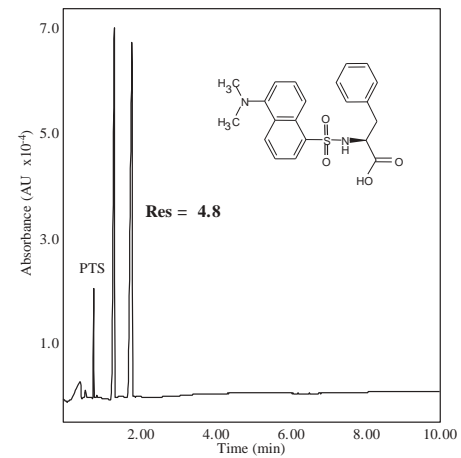
DNS-methionine (10 cm)

HS- γ -CD



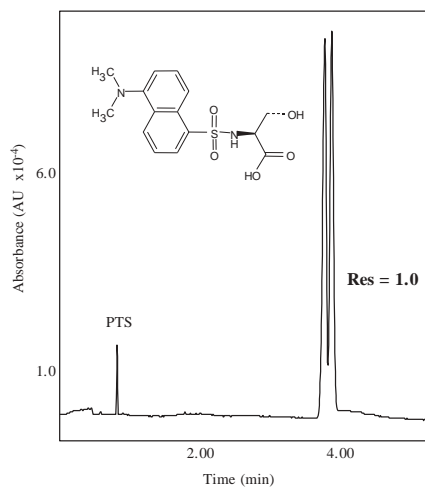
DNS-phenylalanine (10 cm)

HS- γ -CD



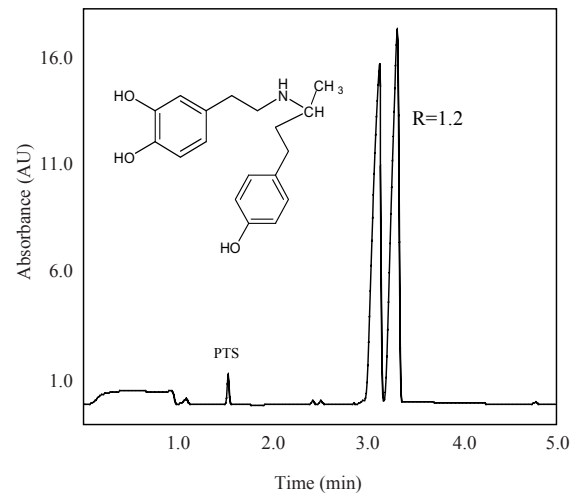
DNS-serine (10 cm)

HS- γ -CD



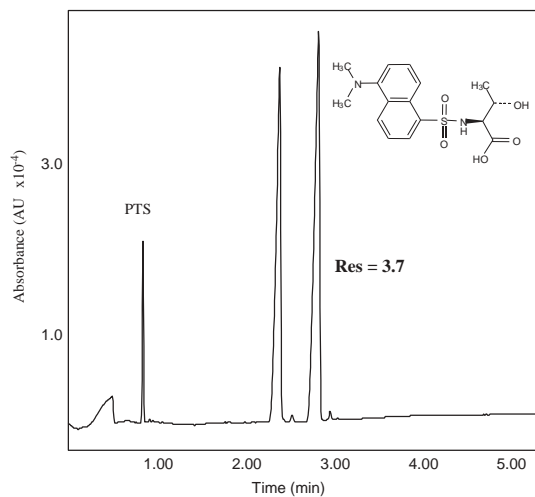
Dobutamine

HS- γ -CD



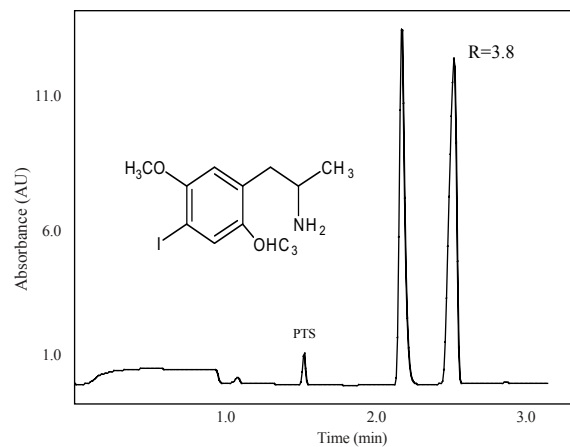
DNS-threonine (10 cm)

HS- γ -CD



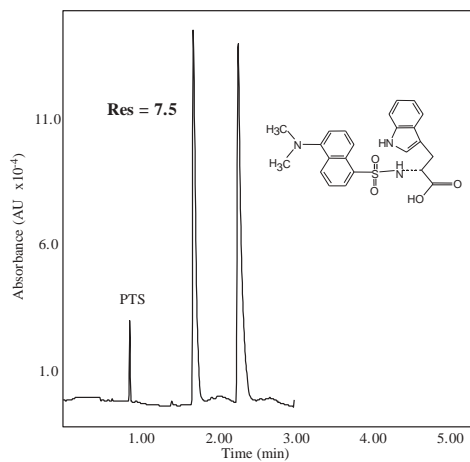
DOI

HS- γ -CD



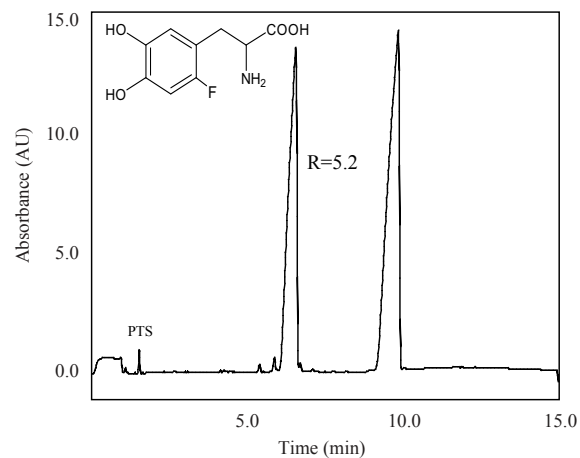
DNS-tryptophan (10 cm)

HS- γ -CD



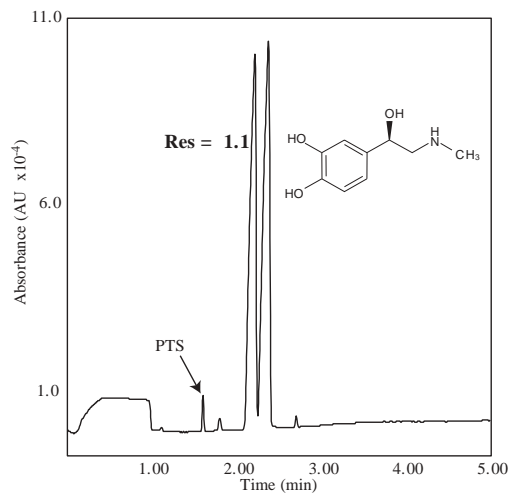
6-F-DOPA

HS- β -CD



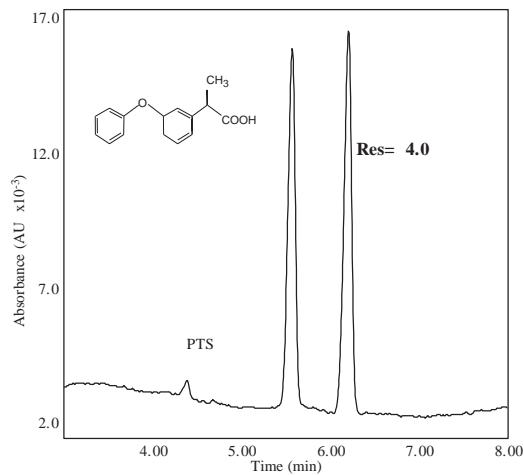
Epinephrine

HS- β -CD



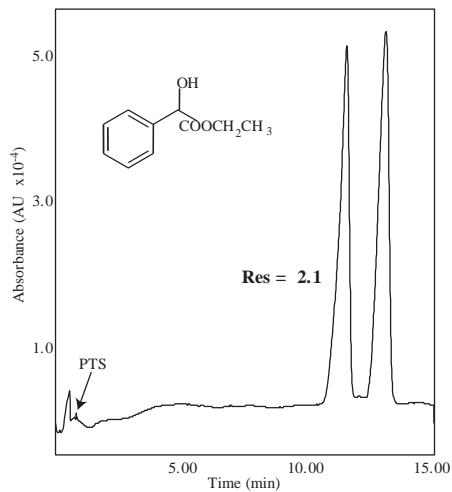
Fenopfenen

HS- β -CD



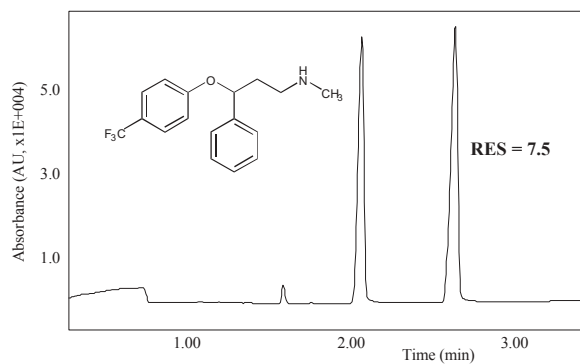
Ethyl mandelate (10 cm)

HS- β -CD



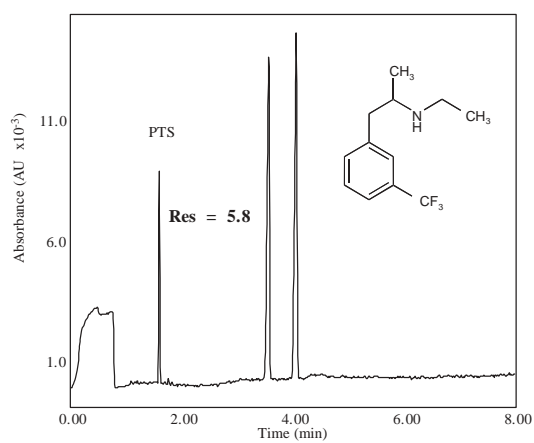
Fluoxetine (Prozac)

HS- γ -CD



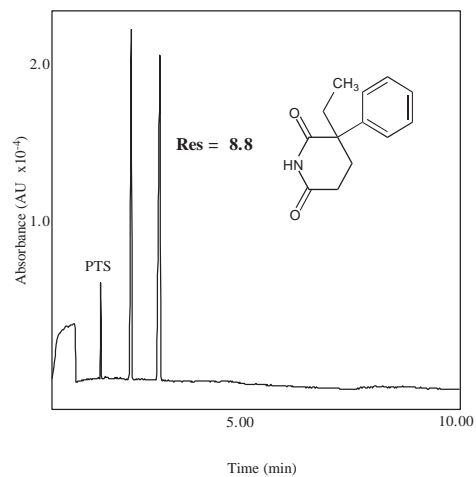
Fenfluramine

HS- β -CD



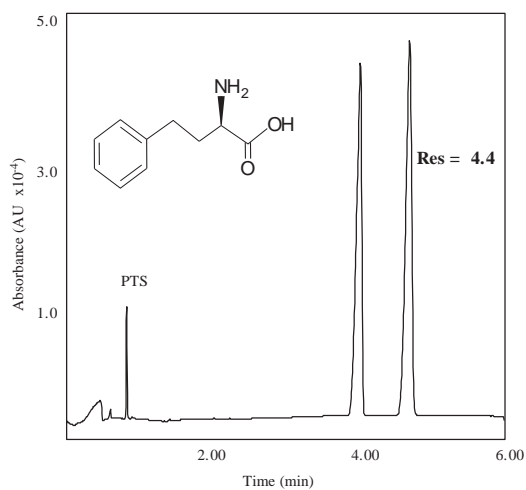
Glutethimide

HS- β -CD



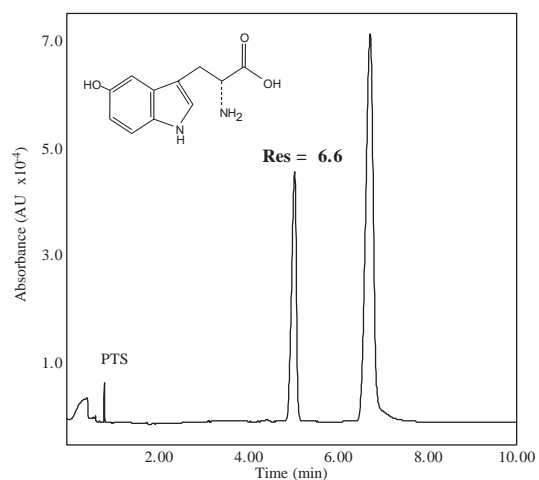
Homophenylalanine (10 cm)

HS- γ -CD



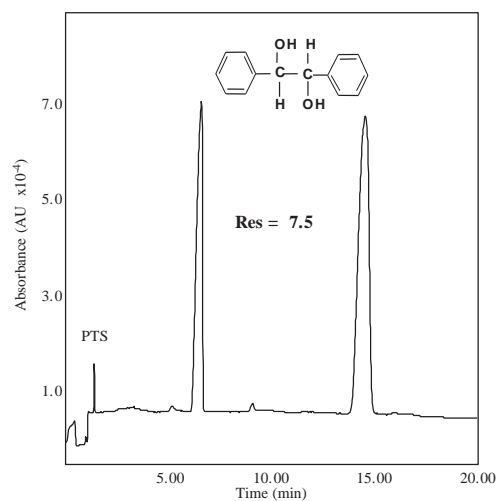
5-Hydroxy-tryptophan (10 cm)

HS- γ -CD



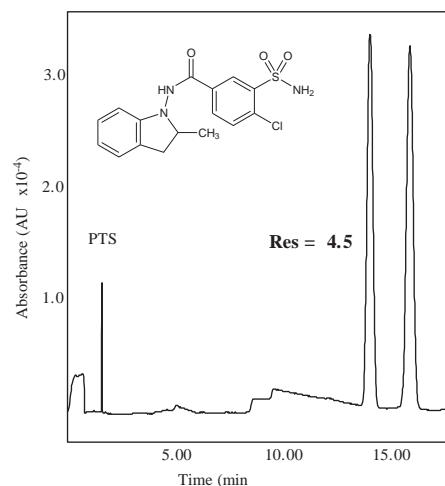
Hydroxybenzoin

HS- β -CD



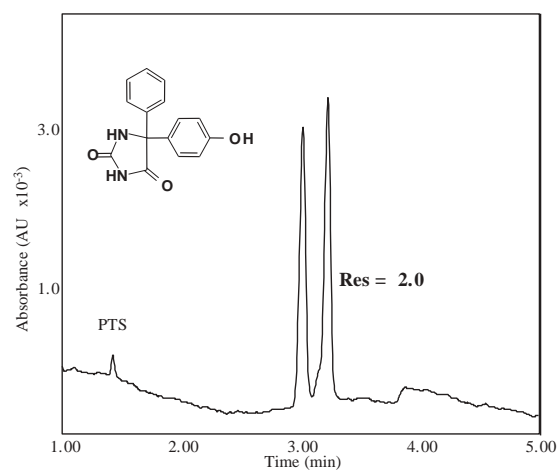
Indapamide

HS- α -CD



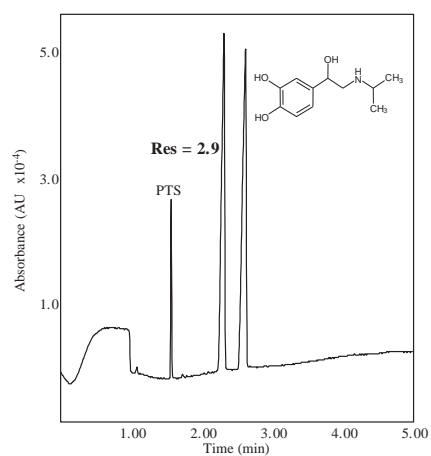
5-(p-Hydroxyphenyl)-5-phenylhydantoin

HS- β -CD



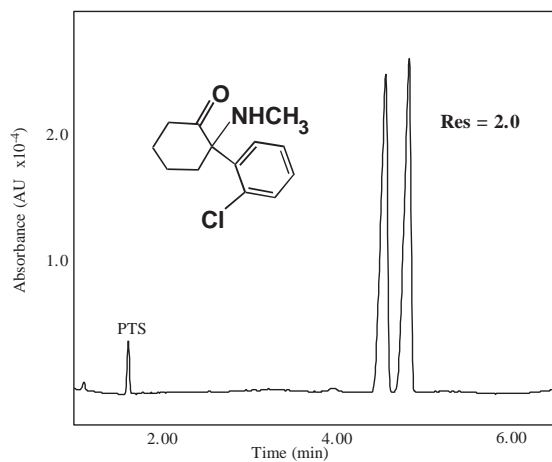
Isoproterenol

HS- β -CD



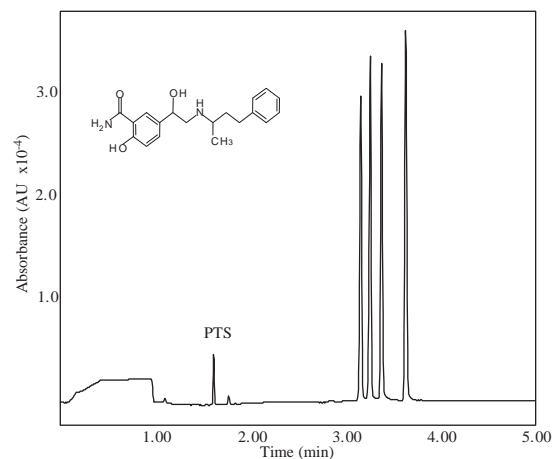
Ketamine

HS- γ -CD



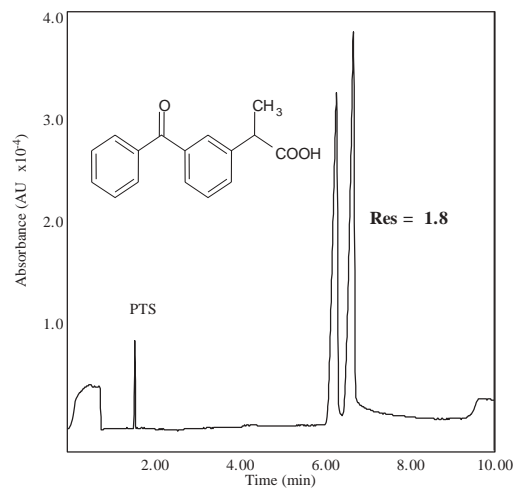
Labetalol

HS- γ -CD



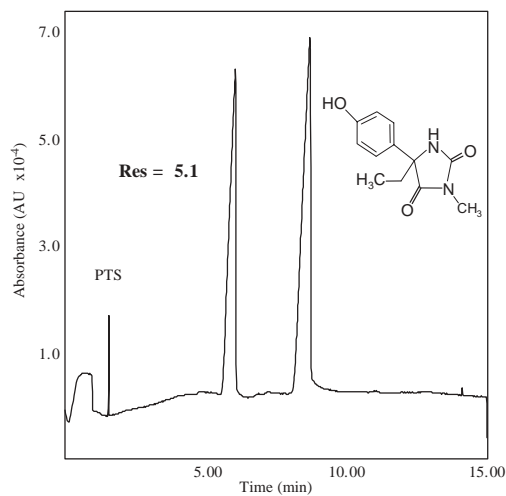
Ketoprofen

HS- β -CD



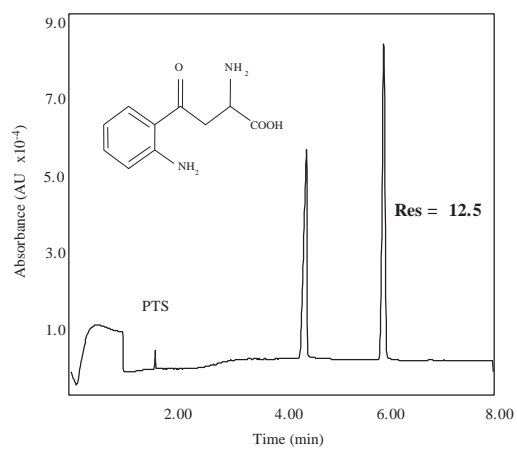
OH-Mephénytoin

HS- β -CD



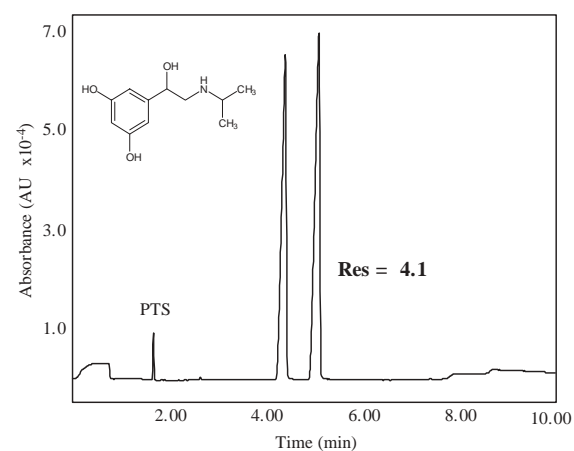
Kynurenine

HS- γ -CD



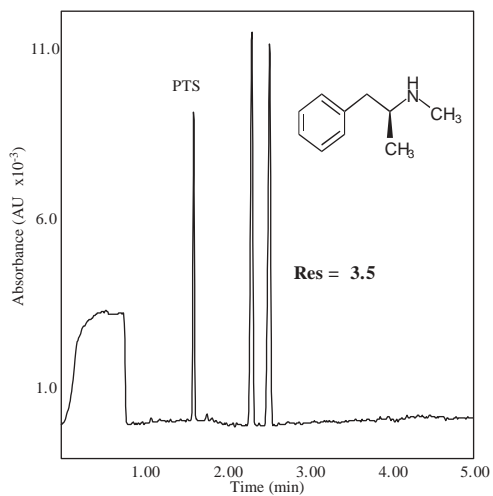
Metaproterenol

HS- γ -CD



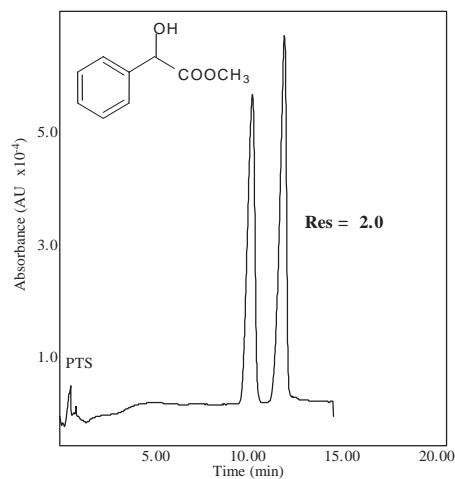
Methamphetamine

HS- β -CD



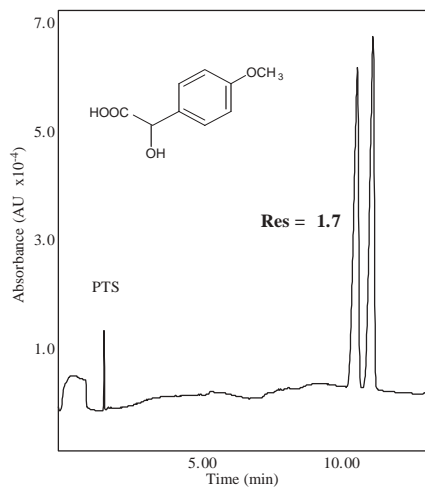
Methyl mandelate (10 cm)

HS- β -CD



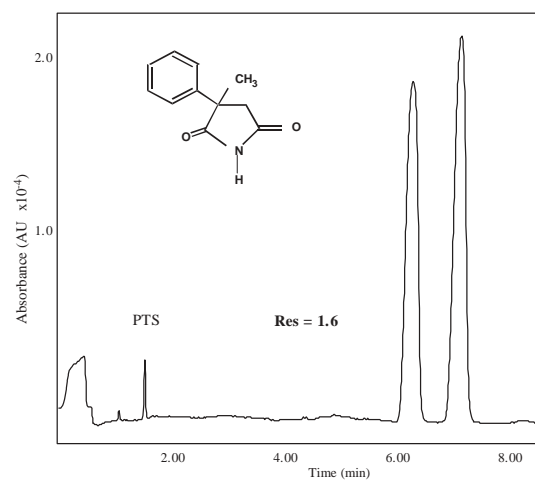
4-Methoxy mandelic acid (10 cm)

HS- β -CD



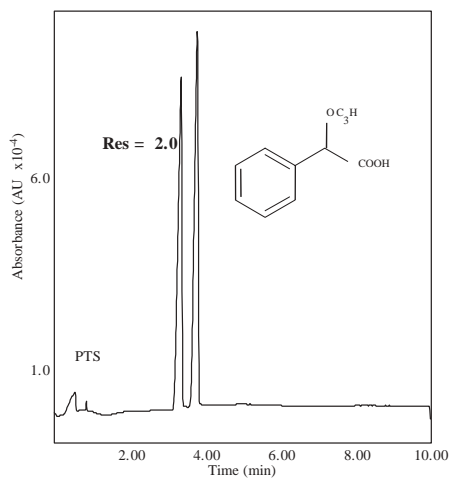
α -Methyl- α -phenylsuccinimide (MPS)

HS- β -CD



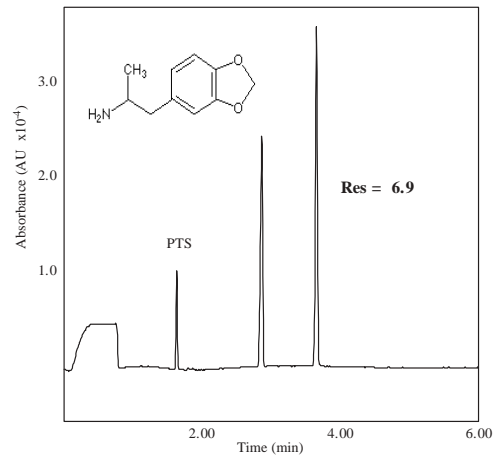
α -Methoxyphenylacetic acid (10 cm)

HS- γ -CD

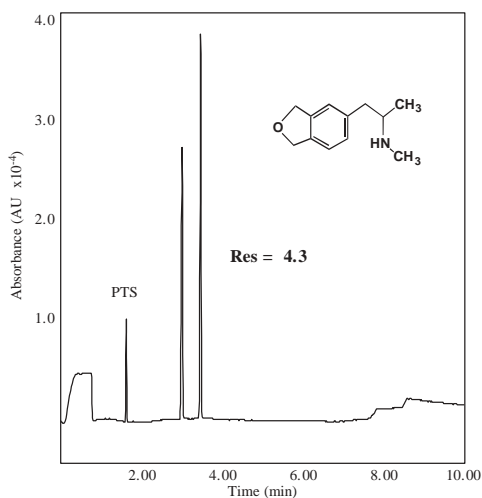


3,4-Methylenedioxyamphetamine (MDMA)

HS- γ -CD

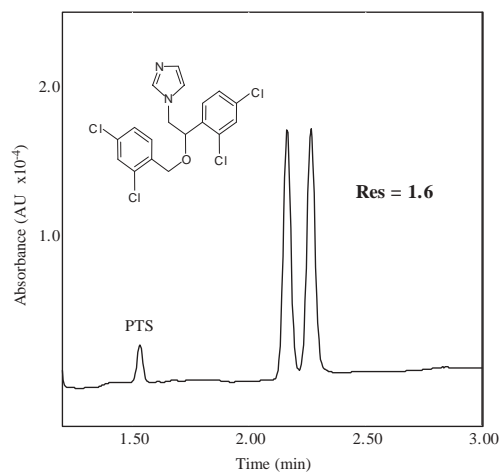


3,4-Methylenedioxyamphetamine (MDMA) HS- γ -CD



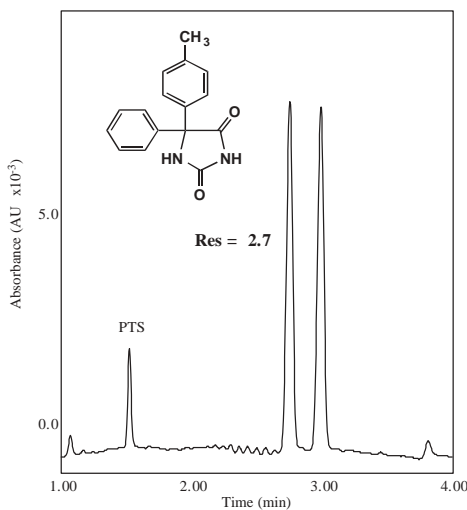
Miconazole

HS- γ -CD



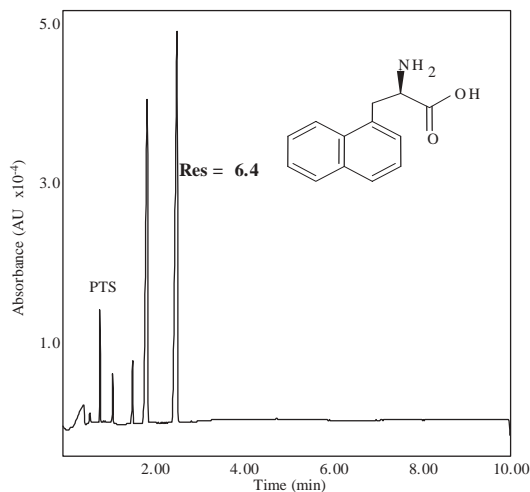
5-(4-Methylphenyl)-5-phenylhydantoin (MPH)

HS- β -CD



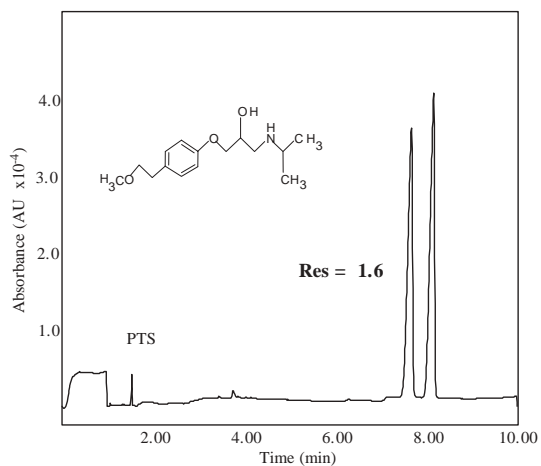
1-Naphthyl alanine (10 cm)

HS- γ -CD



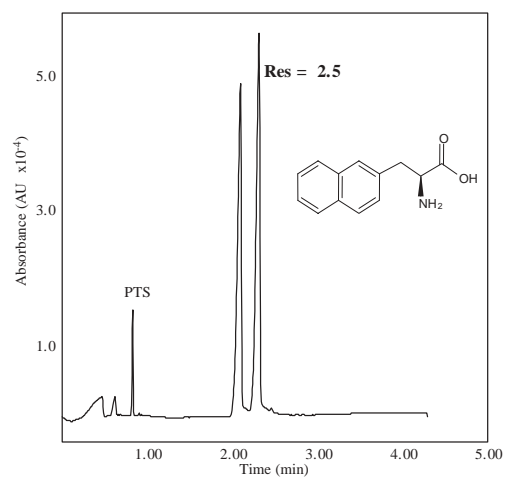
Metoprolol

HS- α -CD



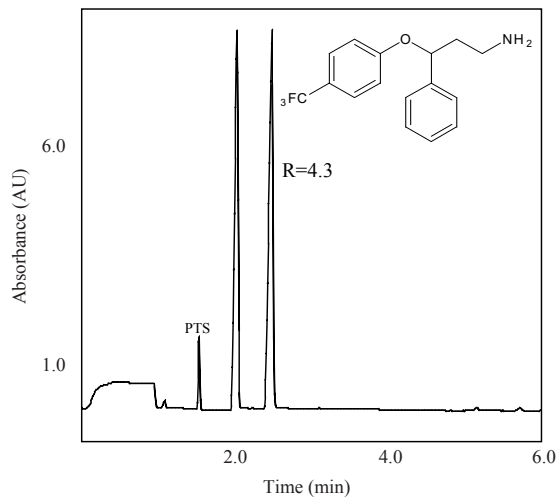
2-Naphthyl alanine (10 cm)

HS- γ -CD



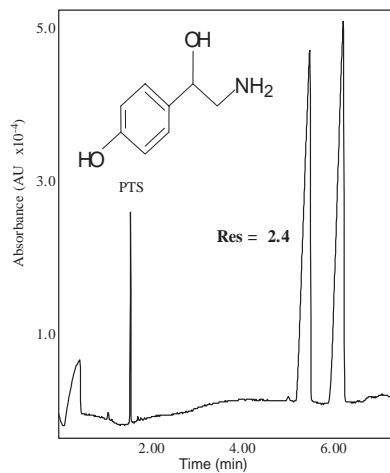
Norfluoxetine

HS- γ -CD



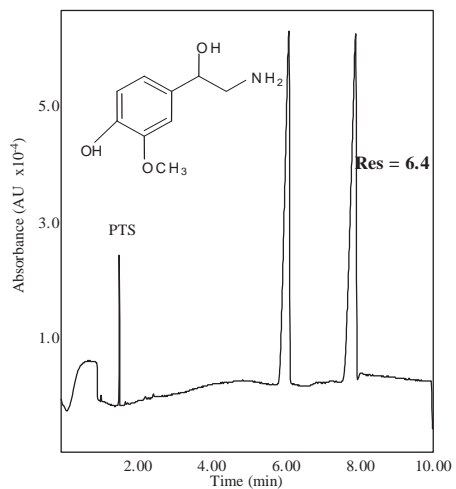
Octopamine

HS- γ -CD



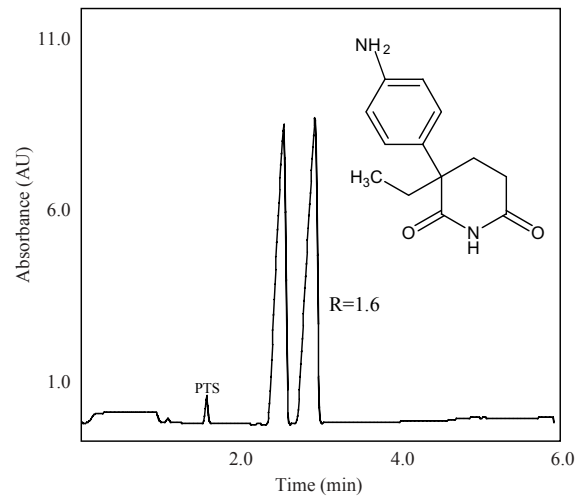
Normetapinephrine

HS- β -CD



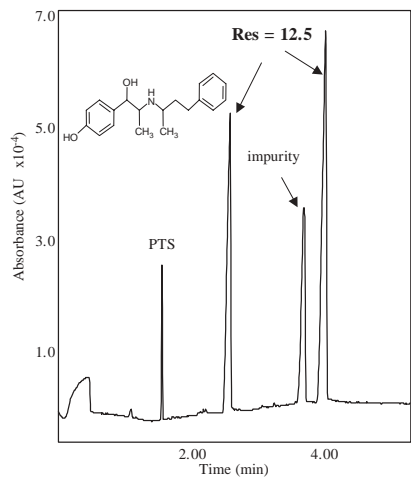
P-Aminogluthethimide

HS- β -CD



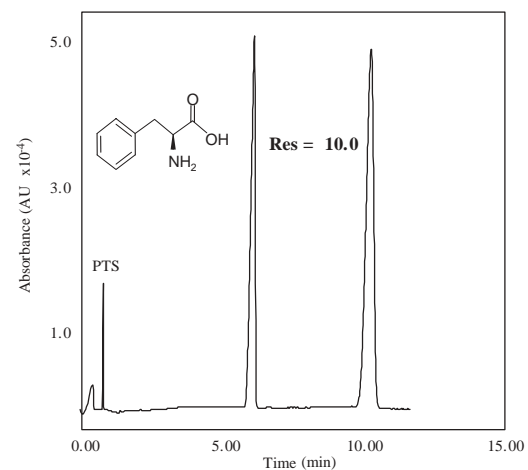
Nylidrin

HS- γ -CD



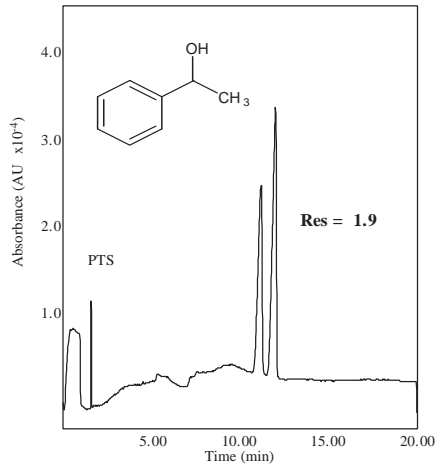
Phenylalanine (10 cm)

HS- γ -CD



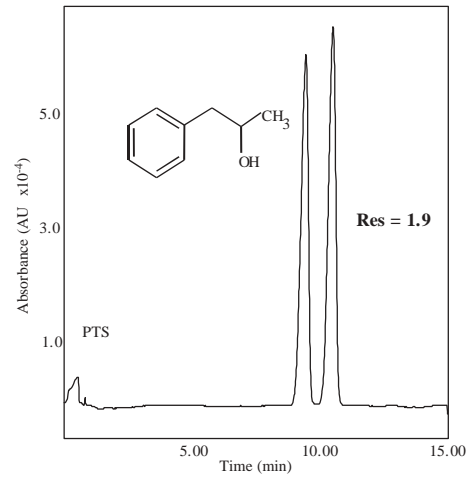
1-Phenylethyl alcohol (10 cm)

HS- β -CD



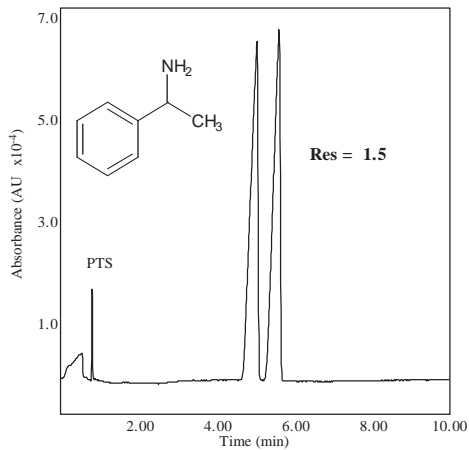
1-Phenyl-2-propanol (10 cm)

HS- γ -CD



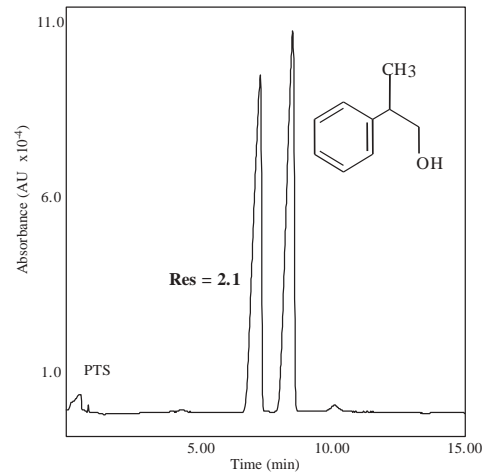
1-Phenylethyl amine (10 cm)

HS- γ -CD



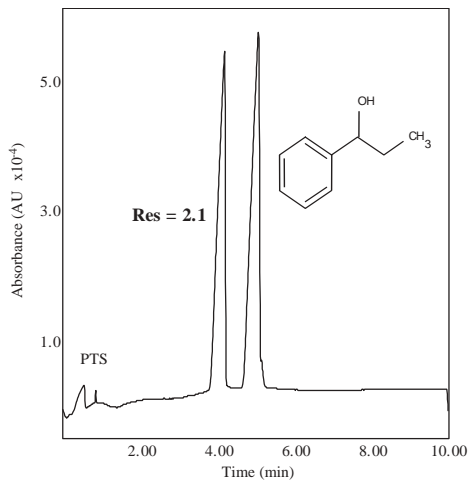
2-Phenyl-1-propanol (10 cm)

HS- γ -CD



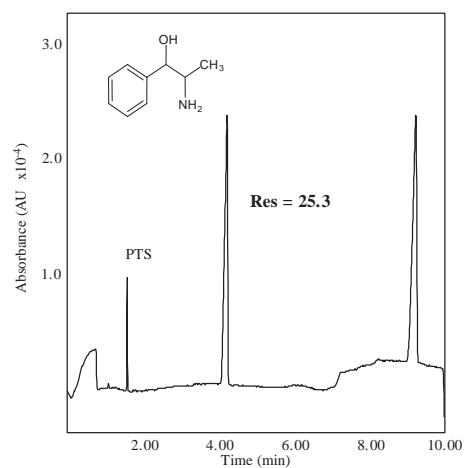
1-Phenyl-1-propanol (10 cm)

HS- β -CD



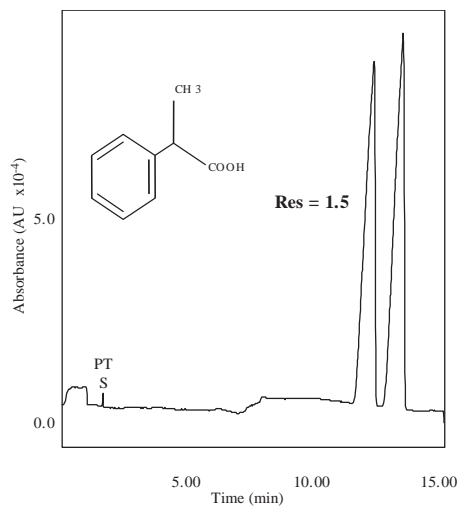
Phenylpropanolamine

HS- γ -CD



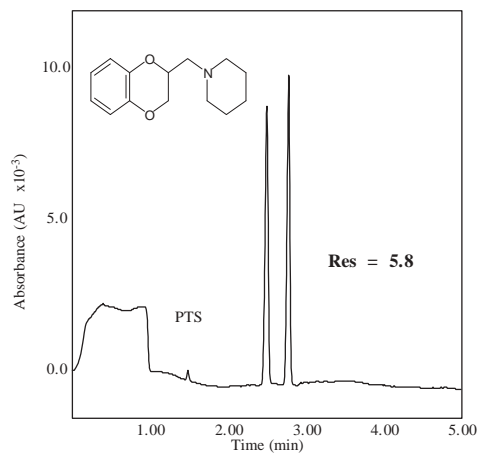
2-Phenyl propionic acid (10 cm)

HS- γ -CD



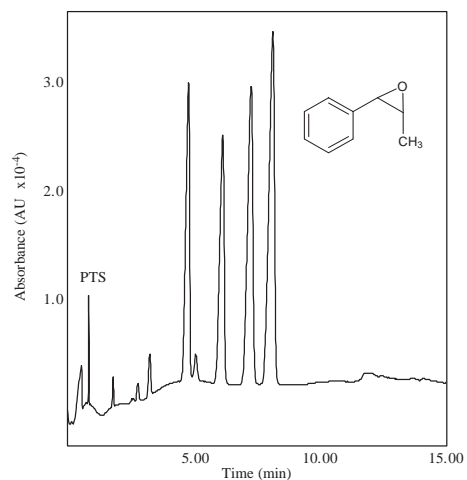
Piperoxan

HS- γ -CD



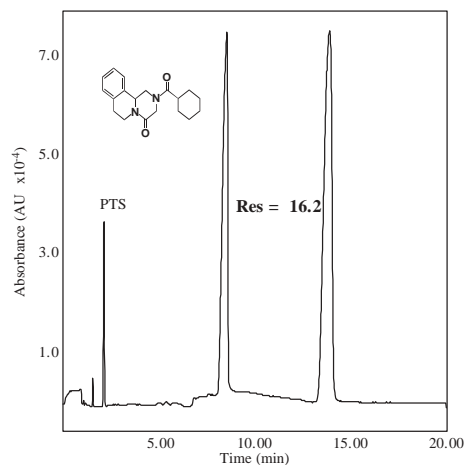
1-Phenylpropylene oxide (10 cm)

HS- β -CD



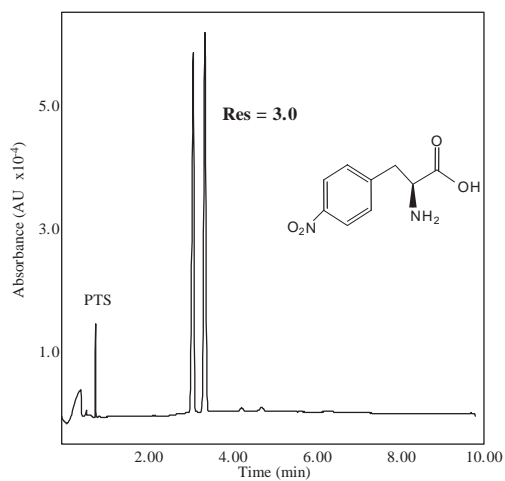
Praziquantel

HS- γ -CD



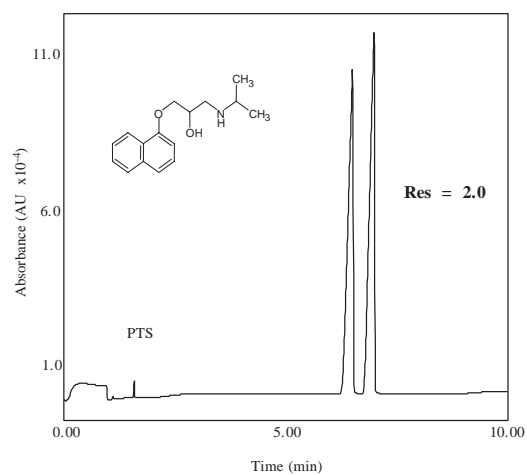
P-Nitro-phenylalanine (10 cm)

HS- β -CD



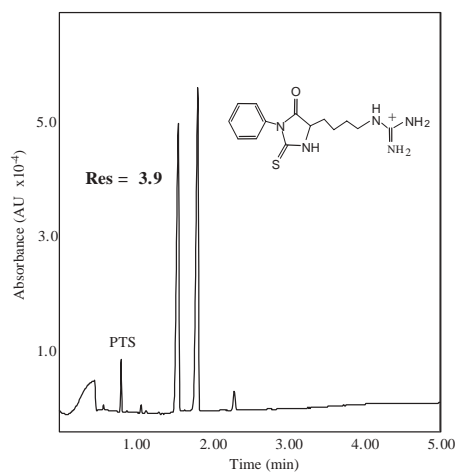
Propranolol

HS- α -CD



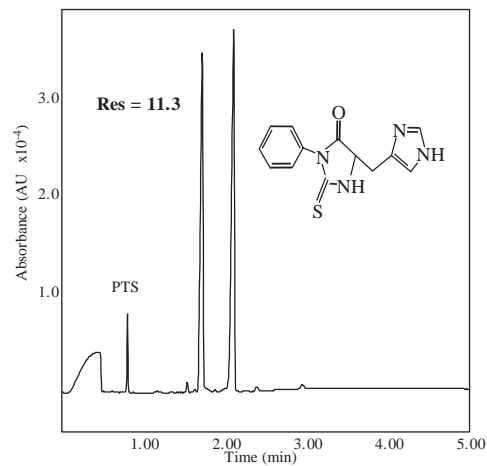
PTH-arginine (10 cm)

HS- β -CD



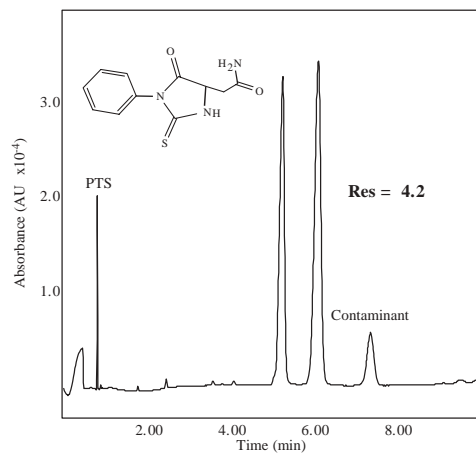
PTH-histidine (10 cm)

HS- β -CD



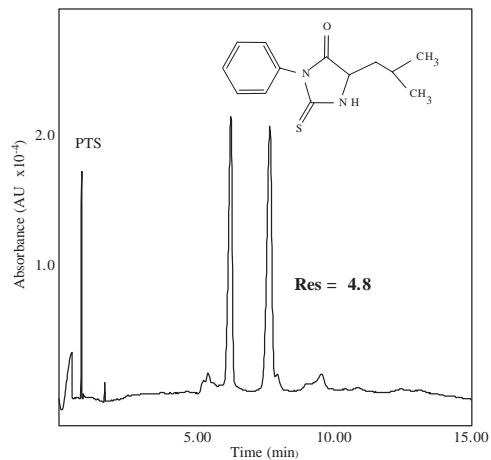
PTH-asparagine (10 cm)

HS- β -CD



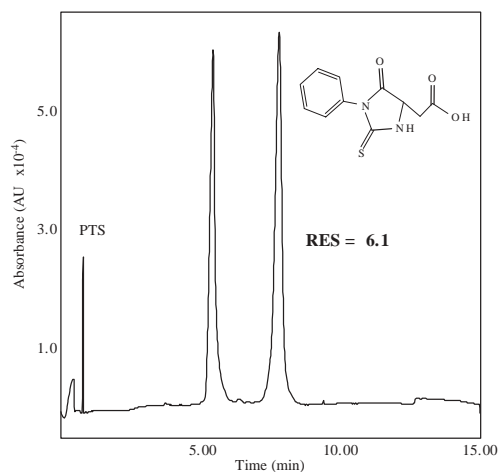
PTH-leucine (10 cm)

HS- β -CD



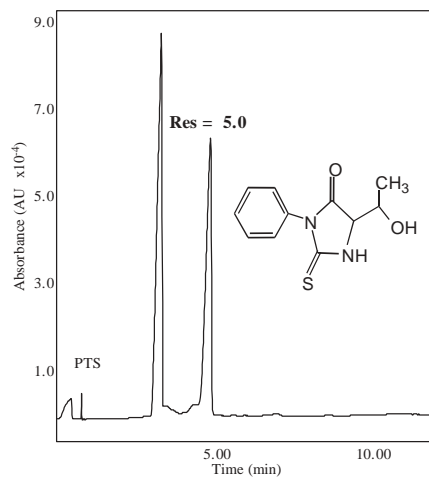
PTH-aspartic acid (10 cm)

HS- β -CD



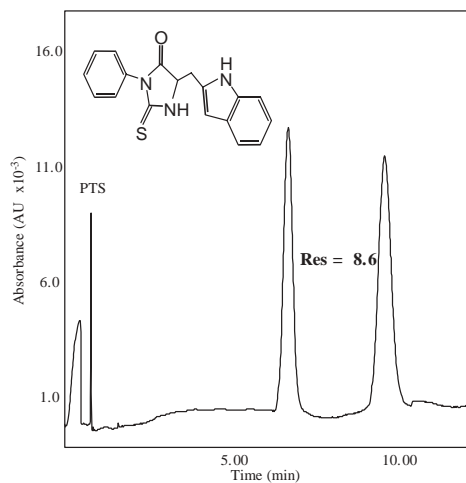
PTH-threonine (10 cm)

HS- β -CD



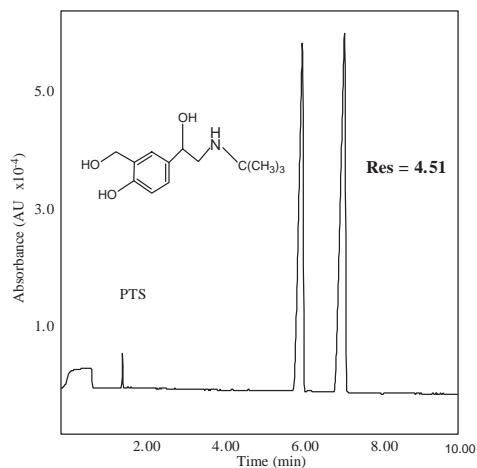
PTH-tryptophan (10 cm)

HS- β -CD



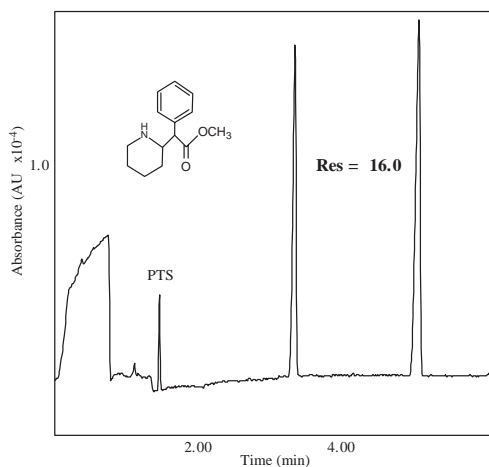
Salbutamol (Proventil)

HS- β -CD



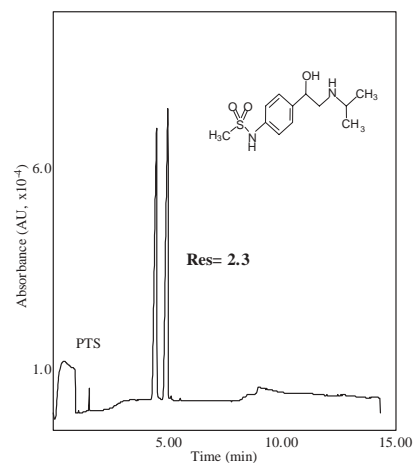
Ritalin

HS- γ -CD



Sotalol

HS- γ -CD

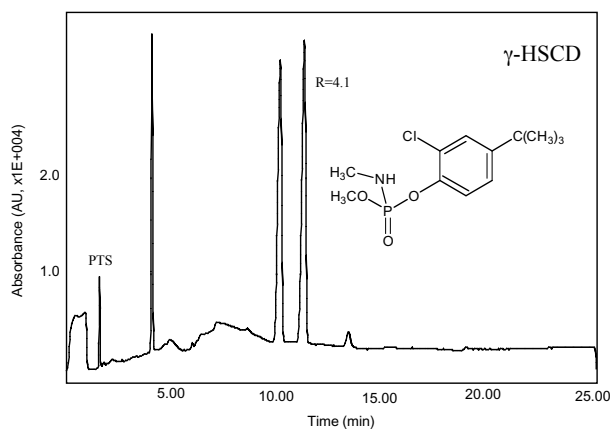


Ruelene

Phosphorus as the chiral center

HS- γ -CD

Readily resolved in HS- γ -CD

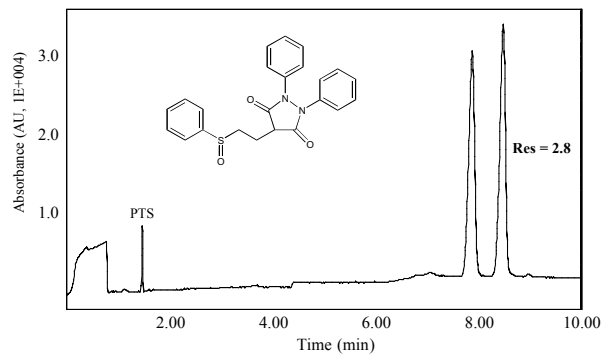


Sulfinpyrazone

Sulfur as the chiral center

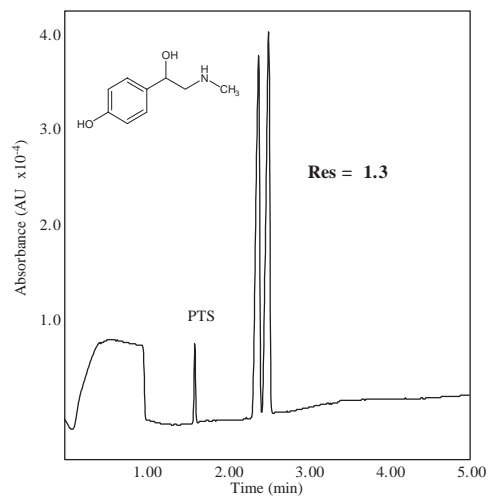
HS- γ -CD

Readily resolved in HS- γ -CD



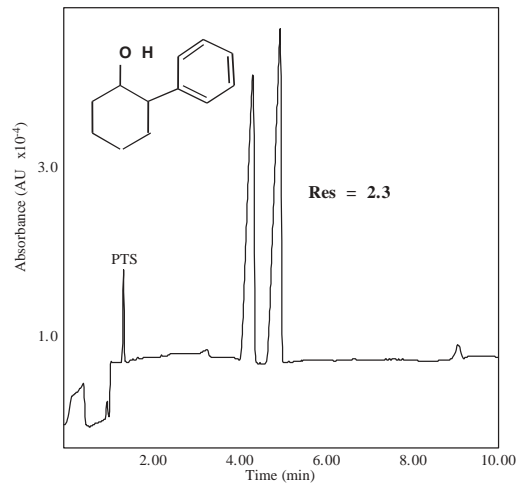
Synephrine

HS- β -CD



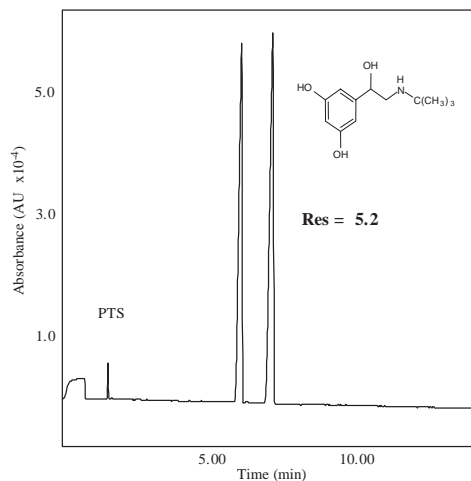
Trans-2-Phenylcyclohexane (TPCH)

HS- β -CD



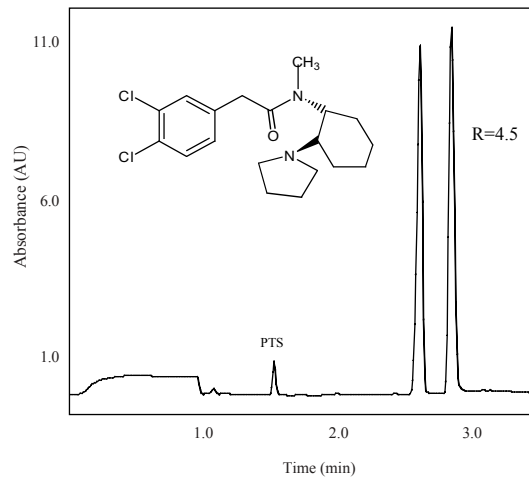
Terbutaline

HS- γ -CD



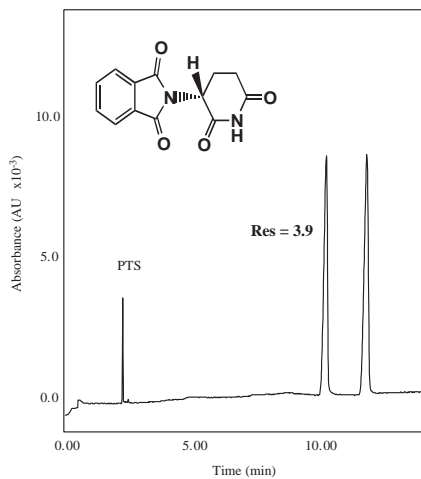
Trans U-50488

HS- γ -CD



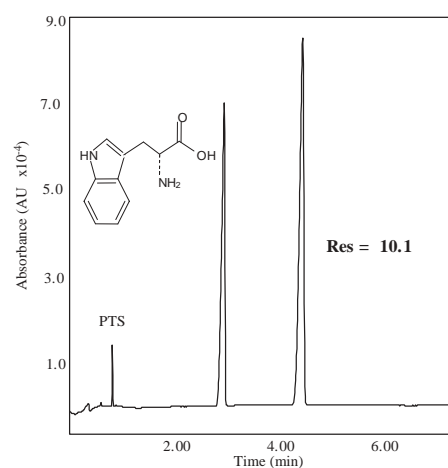
Thalidomide

HS- β -CD



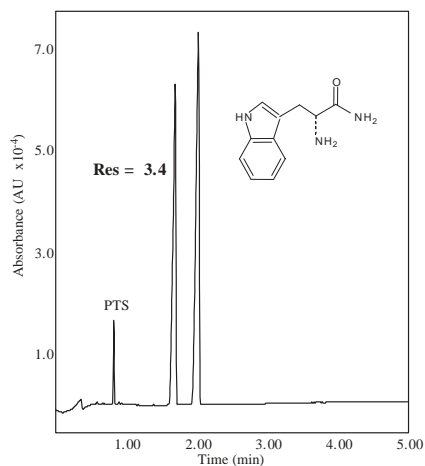
Tryptophane (10 cm)

HS- γ -CD



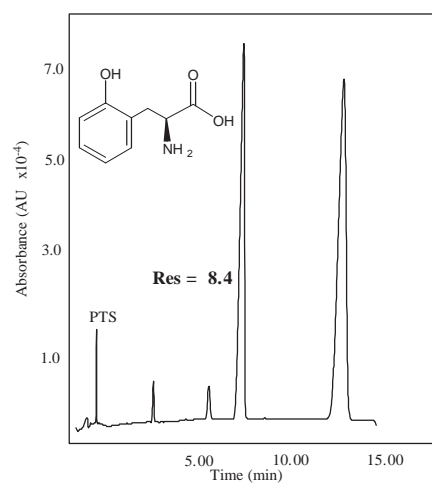
Tryptophanamide (10 cm)

HS- γ -CD



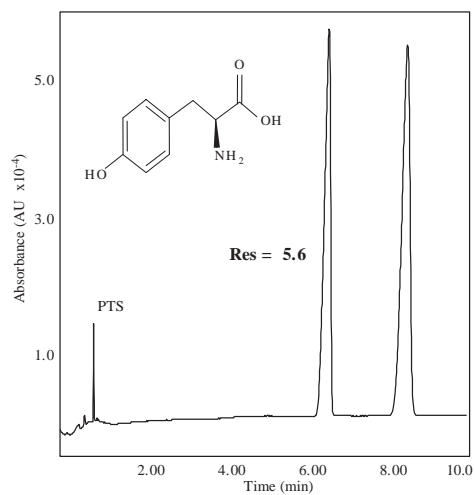
o-Tyrosine (10 cm)

HS- γ -CD



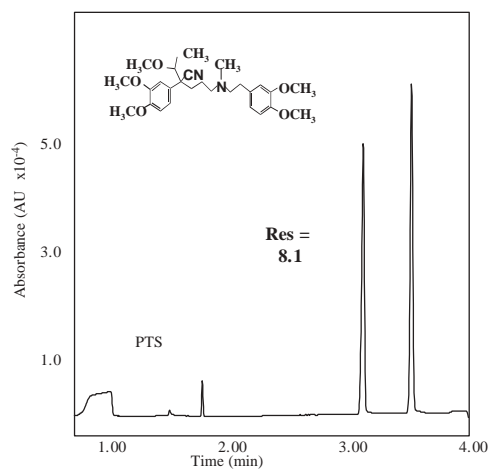
Tyrosine (10 cm)

HS- γ -CD



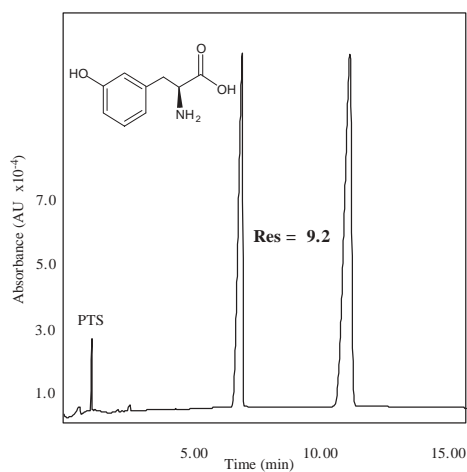
Verapamil

HS- α -CD



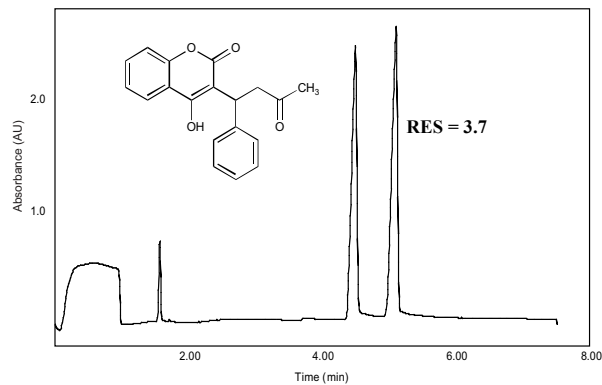
m-Tyrosine (10 cm)

HS- γ -CD



Warfarin

HS- β -CD





Developing innovative solutions in genetic analysis, drug discovery, and instrument systems.

Innovate **Automate**
SIMPLIFY

Beckman Coulter, Inc. • 4300 N. Harbor Boulevard, Box 3100 • Fullerton, California 92834-3100
Sales: 1-800-742-2345 • Service: 1-800-551-1150 • Telex: 678413 • Fax: 1-800-643-4366 • www.beckmancoulter.com

Worldwide Bioresearch Division Offices:

Australia (61) 2 9844-6000 **Canada** (905) 819-1234 **China** (86) 10 6515 6028 **Eastern Europe, Middle East, Africa** (41) 22 994 07 07
France 01 49 90 90 00 **Germany** (89) 358700 **Hong Kong** (852) 2814 7431 / 2814 0481 **Italy** 02-953921 **Japan** 03-5404-8359
Mexico 525-559-16-35 **Netherlands** 0297-230630 **Singapore** (65) 339 3633 **South Africa** (27) 11-805-2014/5 **Spain** (34) 91 3836080
Sweden 08-564 85 900 **Switzerland** 0800 850 810 **Taiwan** (886) 2 2378 3456 **Turkey** 90 216 309 1900 **U.K.** 01494 441181 **U.S.A.** 1-800-742-2345