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Advanced Separation Technologies Inc.

The Influence of Column Temperature on HPLC Chiral Separation on Macrocyclic Glycopeptide CSPs

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Abstract

When comparing macrocyclic glycopeptides, under a given set of conditions, either an increase in resolution or a reversal elution order has been demonstrated for various enantiomers. A study was undertaken to establish what are the conditions required to use temperature as an enhancing operating parameter. The bonded macrocyclic glycopeptide stationary phases used in this study have built within their structure all the well known chiral interactions. What is sometimes lacking is the appropriate bonding distance between the anchor site of the chiral molecule and the chiral interaction mechanism. This situation can often be enhanced at low temperatures.

It has been observed that for the glycopeptide phases strong ionic interactions dominate and the structure of the analyte will determine whether or not an enhancement will occur with a temperature change. Reversal of elution order usually indicates that another mechanism is at work that can be prompted with a temperature change or a change in mobile phase conditions.

This presentation will attempt to identify those conditions where temperature change will initiate a favorable outcome.

Introduction

Temperature has a broad influence on all chromatographic techniques. Its importance is manifested in many ways. For instance, elevated temperatures decrease viscosity and increase solubility and diffusivity. Retention, peak shape, column efficiency, and total analysis time are affected by temperature because both the thermodynamics and kinetics of adsorption processes are functions of temperature. Additionally, temperature control results in improved reproducibility. Although temperature is usually secondary to the selection of column and mobile phase conditions in liquid chromatography (LC), more and more chromatographers begin to consider temperature as imperative to the optimization of a separation.

For chiral separations, selectivity (α) is governed by all types of enantioselective interactions, which are functions of temperature, between analyte and chiral stationary phase (CSP). These interactions include ionic interaction, hydrogen bonding, π - π complexation, dipole stacking, steric interaction and inclusion.

Jetstream Plus

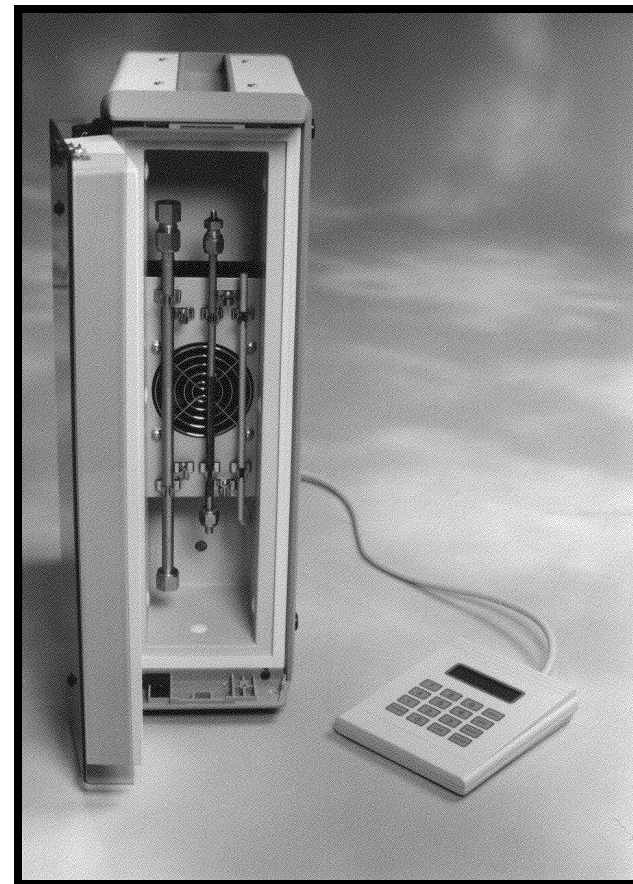
Accurate & Stable Temperature Control

Temperature effects are used mainly for chiral separations, which is hampered by the lack of appropriate instrumentation. **JETSTREAM PLUS** offers full management of temperature enhanced separations, improving the reproducibility and increasing the performance of your analysis. In addition, **JETSTREAM PLUS**, a multiple Peltier column thermostat with heating/cooling capability, has two way forced air circulation. The 5-85°C temperature range is controlled by a new double referenced sensor technology and the auto-calibration feature guarantees reliable results. The setting of fixed temperatures, steps and gradients is done with a simple numerical keypad and a two line character display.

The **JETSTREAM PLUS** Peltier heating/cooling HPLC column thermostat is fully programmable for isothermal, stepwise and linear ramp calculation. Temperature can be input in Celsius or Fahrenheit. The compact unit holds up to 5 columns in lengths up to 40 cm. It includes an integrated leak detector, adjustable sensitivity and acoustic control column protection by temperature lock feature. An RS-232 computer link is included as part of the control circuitry.

Specifications:

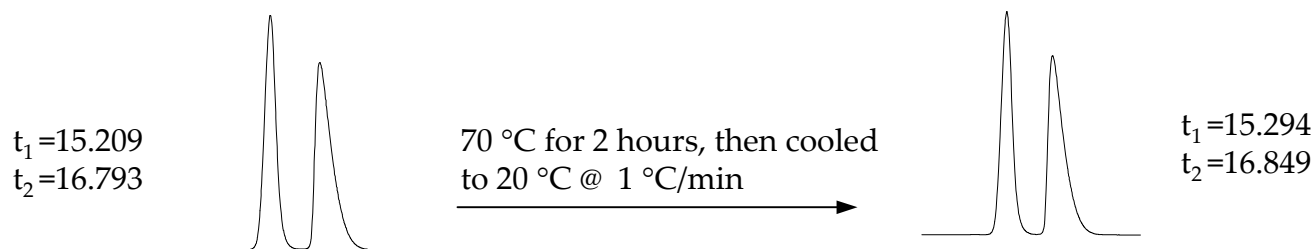
Temp. Range	5-85°C or 0-85°C
Temp. Accuracy	± 0.2°C
Temp. Stability	± 0.1°C
Power	100-245VAC 50/60Hz 100W
Dimensions	135(W)x310(L)x450(H) mm
Weight	8 Kg



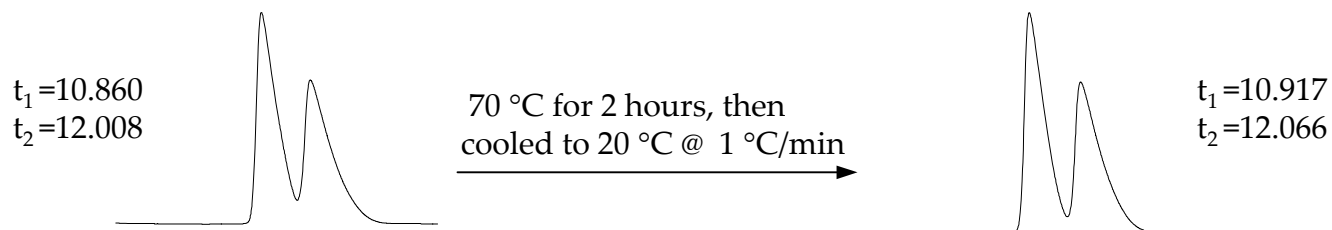
How High Can Column Temperature Go on Chirobiotic Columns?

- The stable, multiple linkages between macrocyclic glycopeptides and silica gel allow the column to be operated at temperatures up to 70°C without any detrimental effect on column performance, but the column temperature should not be changed more than 2°C /min!

Polar Ionic Mode (Analyte: N-benzyl- α -methylbenzylamine; Column: Chirobiotic V, 4.6 x 250 mm; Mobile Phase: MeOH/HOAc/TEA 100/0.02/0.01, v/v/v; Flow rate: 1.0 ml/min, Detector: UV@254 nm; 20 °C



Reversed Phase Mode (Analyte: N-benzyl- α -methylbenzylamine; Column: Chirobiotic V, 4.6 x 250 mm; Mobile Phase: MeOH/0.1%TEA 30/70 v/v; Flow rate: 1.0 ml/min; Detector: UV@ 254 nm; 20 °C



Temperature Effect on Retention

- For most compounds, higher temperature results in shorter retention time.

$$\ln(k') = -\Delta H^\circ/RT + \Delta S^\circ/R + \ln(\phi)$$

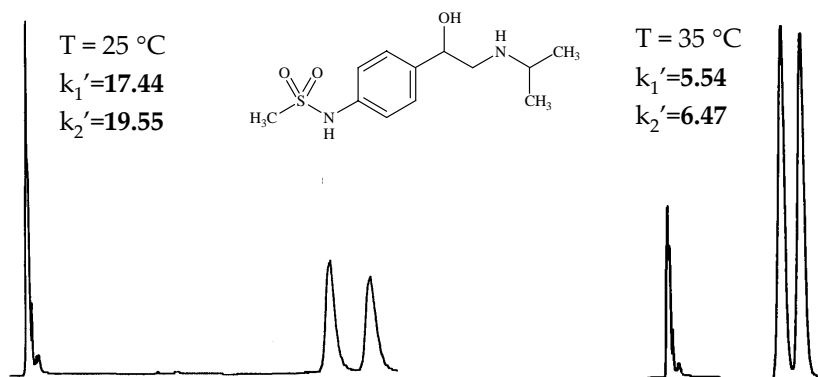
Compound: Sotabol

Column: Chirobiotic TAG (4.6 mm x 250 mm)

Mobile phase: MeOH/HOAc/TEA 100/0.1/0.1 (v/v/v)

Flow rate: 0.9 ml/min

Detection: UV@ 230 nm



- However, there exist exceptions.

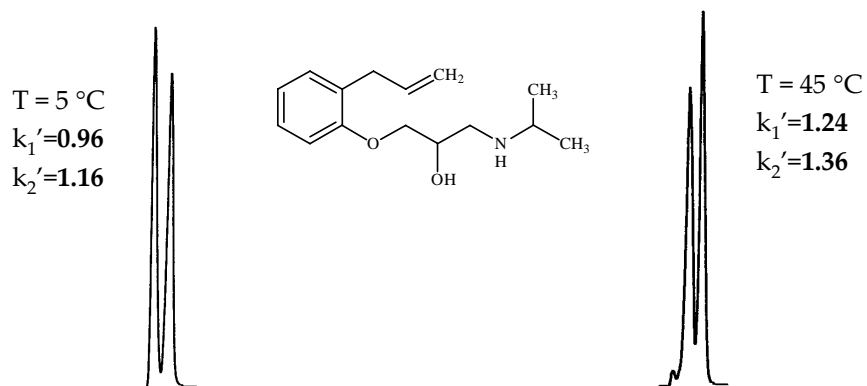
Compound: Alprenolol

Column: Chirobiotic V (4.6 mm x 250 mm)

Mobile phase: MeOH/HOAc/TEA 100/0.1/0.1 (v/v/v)

Flow rate: 0.9 ml/min

Detection: UV@ 230 nm



Temperature Effect on Peak Efficiency

- High temperature typically results in high peak efficiency.

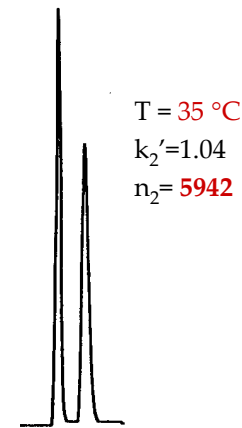
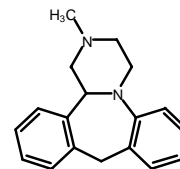
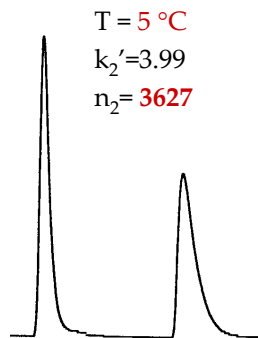
Compound: Mianserin

Column: Chirobiotic V (4.6 mm x 250 mm)

Mobile phase: MeOH/HOAc/TEA 100/0.1/0.1 (v/v/v)

Flow rate: 0.9 ml/min

Detection: UV@ 230 nm



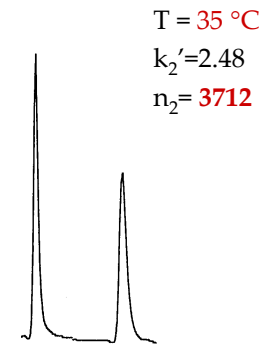
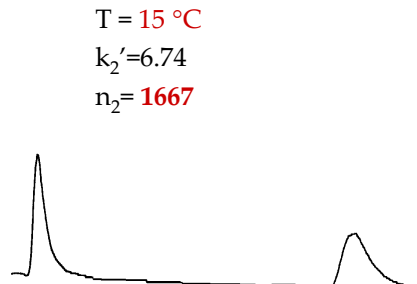
Compound: 3-Indolelactic acid

Column: Chirobiotic R (4.6 mm x 250 mm)

Mobile phase: MeOH/0.1% TEAA (pH4.1) 40/60 v/v

Flow rate: 1.0 ml/min

Detection: UV@ 230 nm



Temperature Effect Showing On-Column Racemization

- The phenomena of on-column racemization can be eliminated by lowering the column temperature

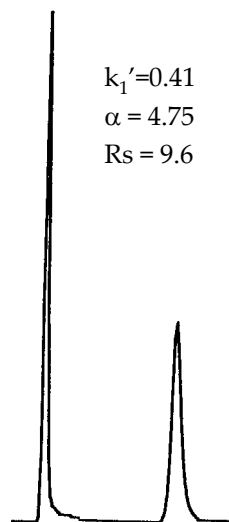
Compound: Oxazepam

Column: Chirobiotic T (4.6 mm x 250 mm)

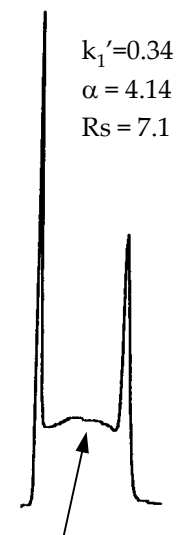
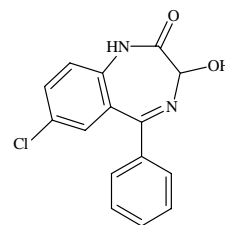
Mobile phase: MeOH/HOAc/TEA 100/0.2/0.2 v/v/v

Flow rate: 0.9 ml/min

Detection: UV@ 230 nm



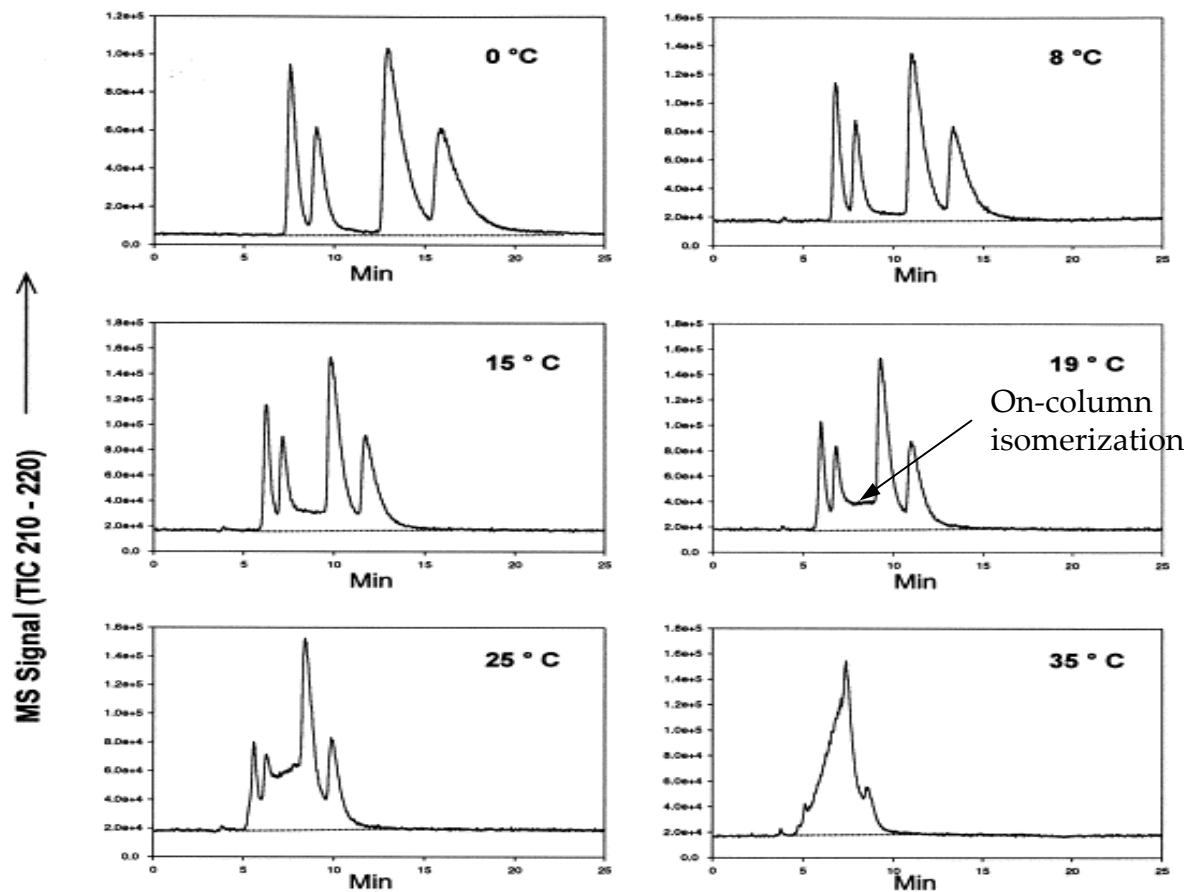
15 °C



On-column racemization

35 °C

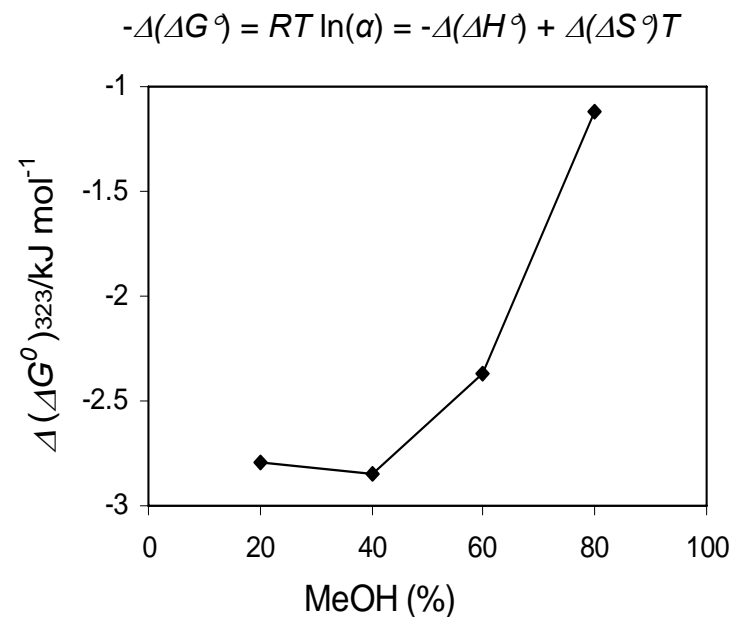
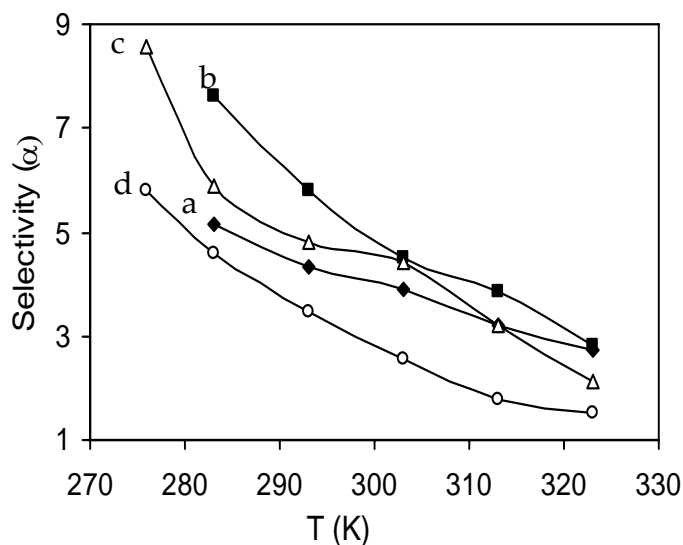
Temperature Study During Method Development



Compound: Captopril diastereoisomers
 Column: Chirobiotic T (4.6 mm x 250 mm)
 Mobile phase: 0.05% TEAA (pH3.8) buffer
 Flow rate: 1.0 ml/min
 Detection: mass spectrometer in negative ion mode scanning between 210 and 220 mass units

Optimize Separation Conditions Before Temperature Study

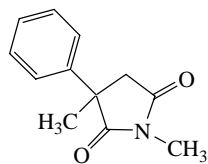
- Mobile phase conditions must be optimized before investigating the effects of temperature.



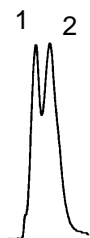
Compound: N-carbobenzyloxy-D,L-tryptophan (CBZ-Trp); Column: Chirobiotic R (250 x 4.6 mm); Flow rate: 1 ml/min; Detection: UV @ 205 nm; Mobile Phase: a) MeOH/0.1%TEAA (pH6.5) 20/80 (v/v); b) MeOH/0.1%TEAA (pH6.5) 40/60 (v/v); c) MeOH/0.1%TEAA (pH6.5) 60/40 (v/v); d) MeOH/0.1%TEAA (pH6.5) 80/20 (v/v). Adapted from Antal Peter, *et. al.* J. Chromagr. A, 958, (2002), 89-107. Courtesy of Antal Peter.

Reverse Elution Order on Chirobiotic Columns

Compound: Methsuximide;
Mobile phase: EtOH/Hex 10/90;
Flow rate: 0.9 ml/min; Detector:
UV @ 230 nm



Chirobiotic V



5 °C



15 °C



20 °C

Chirobiotic T



5 °C



10 °C



15 °C

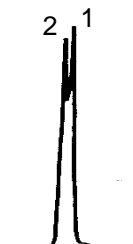
Chirobiotic R



15 °C

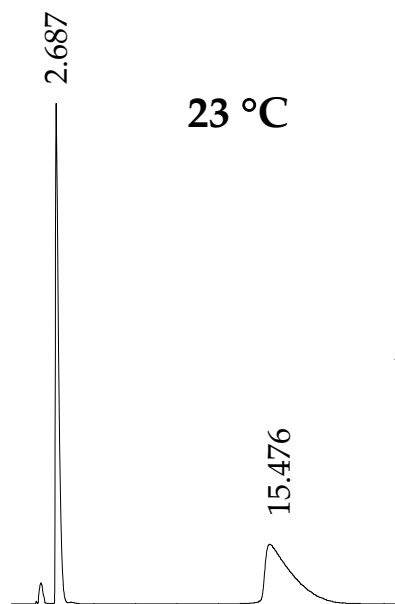


25 °C



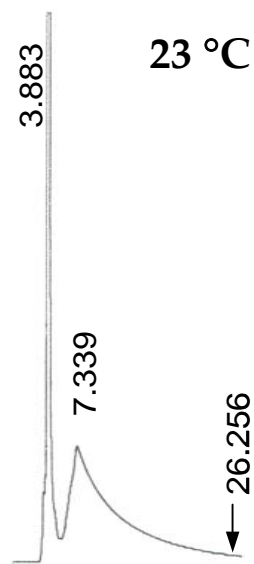
35 °C

Application of Temperature Effect in Loading Study of Diacetyl-Cysteine



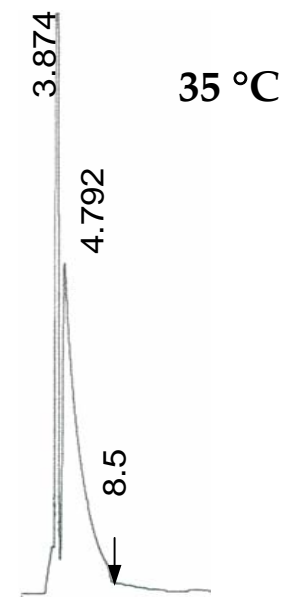
Column: Chirobiotic T (4.6x250 mm)
Mobile Phase: MeOH/ NH₄OAc
100/0.1% (v/w)
Flow Rate: 2.0 ml/min
Detector: UV@ 230 nm

Semi-Prep →



Loading: 15 mg
Column: Chirobiotic T (4.6x250 mm)
Mobile Phase: MeOH/ NH₄OAc
100/1.0% (v/w)
Flow Rate: 1.0 ml/min
Detector: UV@ 230 nm

Higher Temp →



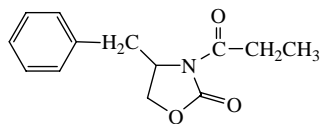
Loading: 15 mg
Column: Chirobiotic T (4.6x250 mm)
Mobile Phase: MeOH/ NH₄OAc
100/0.1% (v/w)
Flow Rate: 1.0 ml/min
Detector: UV@ 230 nm

Less Time!

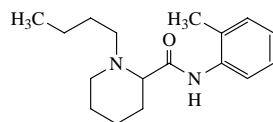
Less mobile phase additive!

Molecules for the Study of Temperature Effect on Chirobiotic V

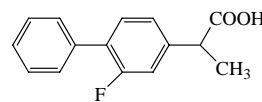
(a) 4-Benzyl-3-propionyl-2-oxazolidinone



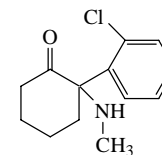
(b) Bupivacaine



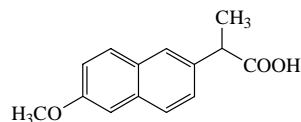
(c) Flurbiprofen



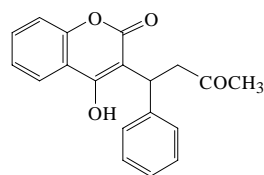
(d) Ketamine



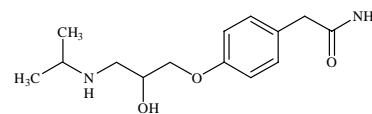
(e) Naproxen



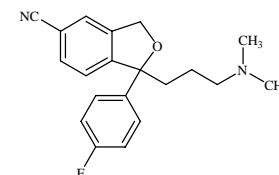
(f) Warfarin



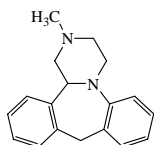
(g) Atenolol



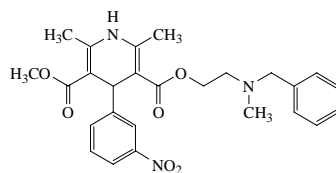
(h) Citalopram



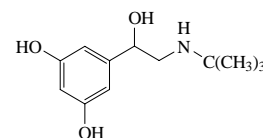
(i) Mianserin



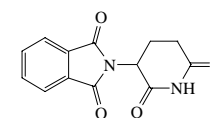
(j) Nicardipine



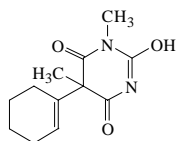
(k) Terbutaline



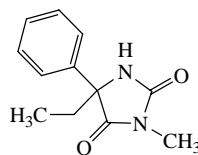
(l) Thalidomide



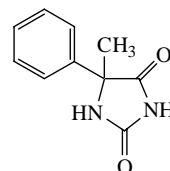
(m) Hexobarbital



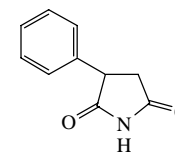
(n) Mephentoin



(o) 5-Methyl-5-phenylhydantoin

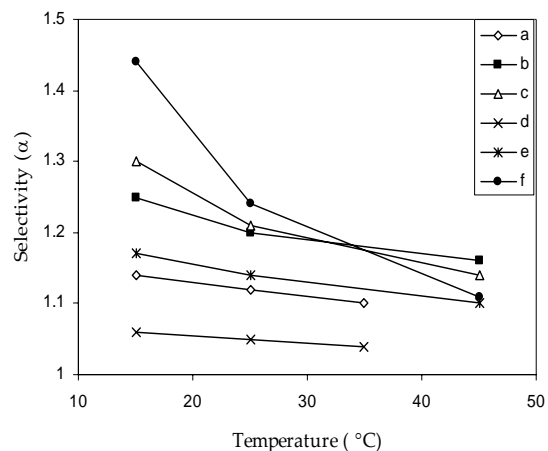


(p) Phensuximide



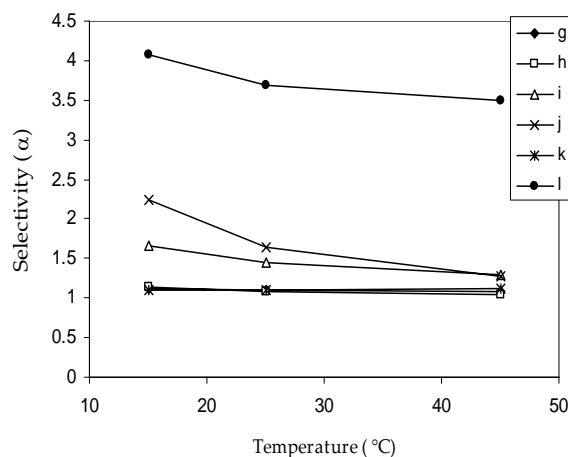
Temperature Effect on Selectivity (α) Chirobiotic V

Reversed Phase Mode



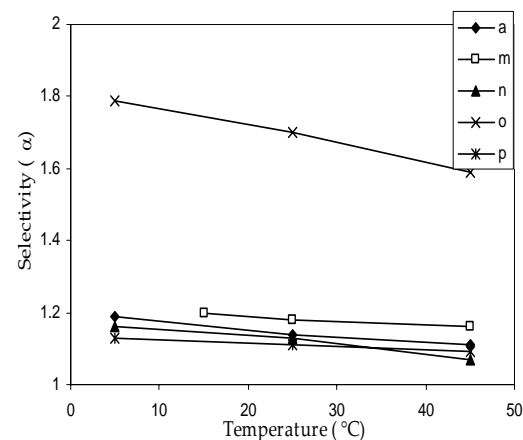
Mobile phase: for a), THF/20 mM NH_4NO_3 (pH 5.5) 30/70 v/v; for b), c) and e), THF/ 20 mM sodium citrate (pH 6.3) 10/90 v/v; for d), MeOH/0.1% TEAA (pH 4.1) 30/70 v/v; for f) THF/0.1% TEAA (pH 6.0) 20/80, v/v; Flow rate: 0.9 ml/min; Detection: UV@230 nm.

Polar Ionic Mode[®]



Mobile phase: for g), h), i), and k), MeOH/HOAc/TEA 100/0.1/0.1 v/v/v; for j) and l), MeOH/HOAc/TEA 100/0.01/0.01 v/v/v; Flow rate: 0.9 ml/min; Detection: UV@230 nm.

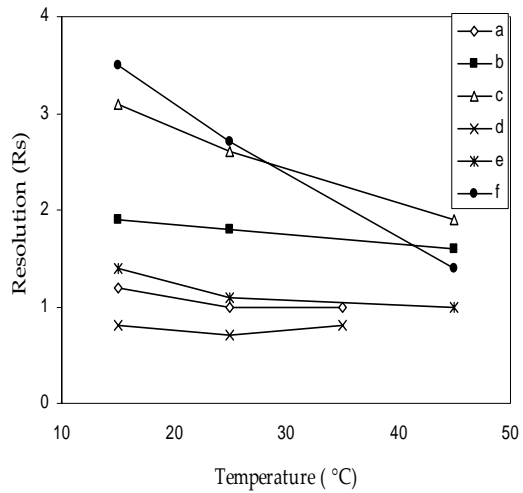
Normal Phase Mode



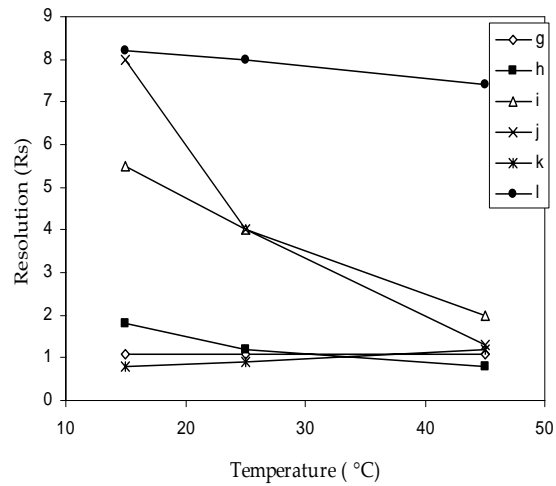
Mobile phase: EtOH/Hex 50/50; flow rate: 1.0 ml/min; Detection: UV@254 nm.

Temperature Effect on Resolution (R_s) Chirobiotic V

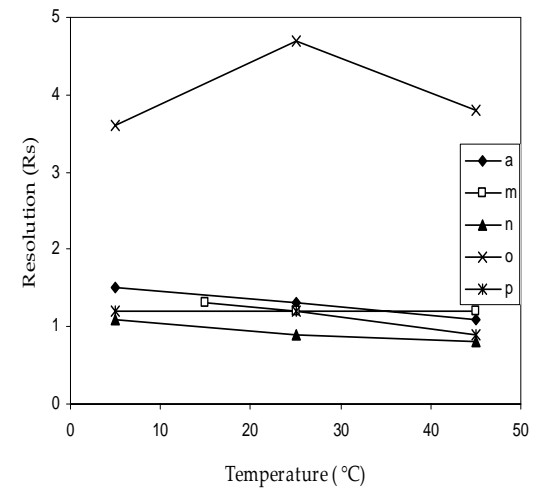
Reversed Phase Mode



Polar Ionic Mode[®]

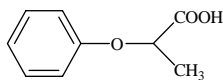


Normal Phase Mode

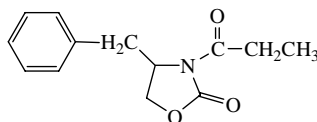


Molecules for the Study of Temperature Effect on Chirobiotic T

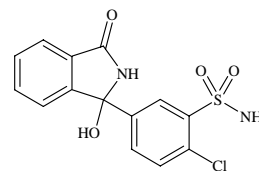
(a) 2-Phenoxypropionic acid



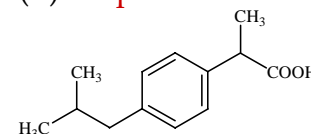
(b) 4-Benzyl-3-propionyl-2-oxazolidinone



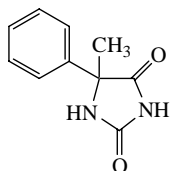
(c) Chlorthalidone



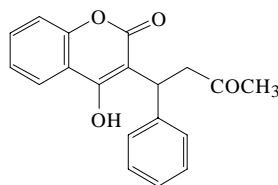
(d) Ibuprofen



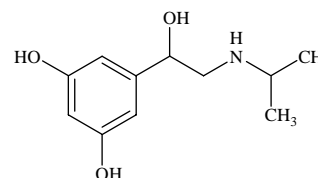
(e) 5-Methyl-5-phenylhydantoin



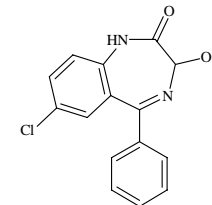
(f) Warfarin



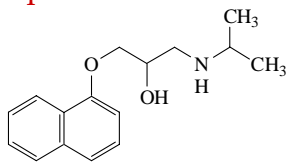
(g) Metaproterenol



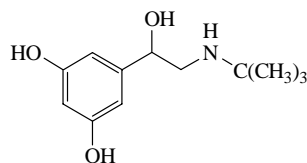
(h) Oxazepam



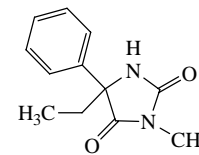
(i) Propranolol



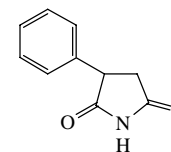
(j) Turbutaline



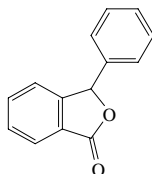
(k) Mephentoin



(l) Phensuximide

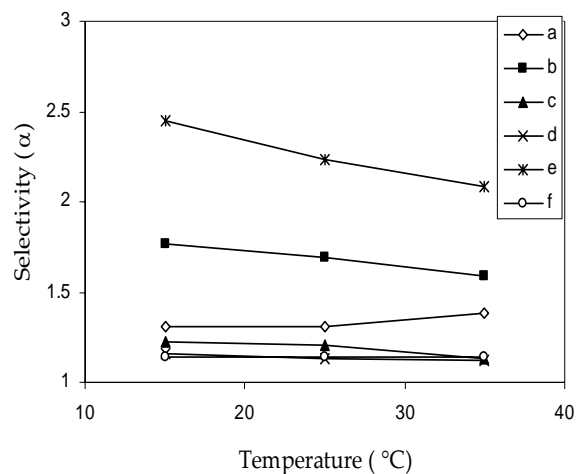


(m) 3-Phenylphthalide



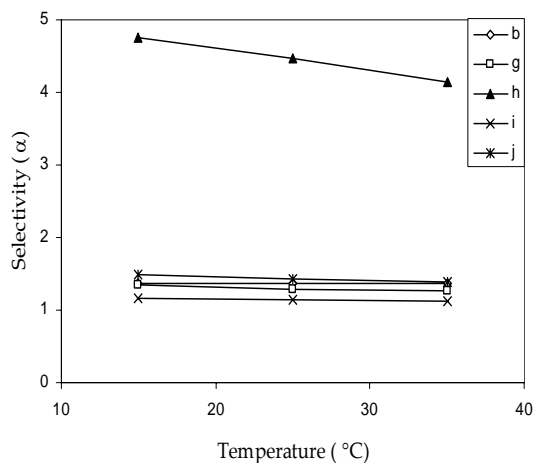
Temperature Effect on Selectivity (α) Chirobiotic T

Reversed Phase Mode



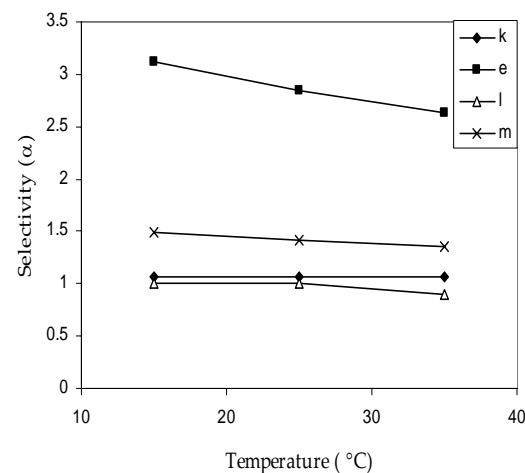
Mobile phase: MeOH/0.1% TEAA
(pH 4.1) 30/70; Flow rate: 0.9
ml/min; Detection: UV@230 nm.

Polar Ionic Mode[®]



Mobile phase: MeOH/HOAc/TEA
100/0.2/0.2 v/v/v; Flow rate: 0.9
ml/min; Detection: UV@230 nm.

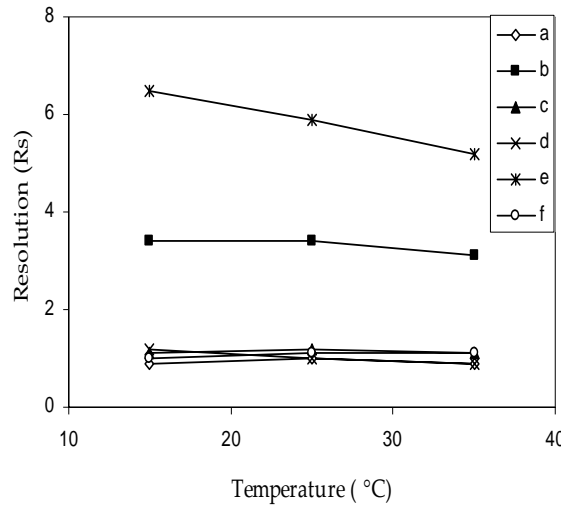
Normal Phase Mode



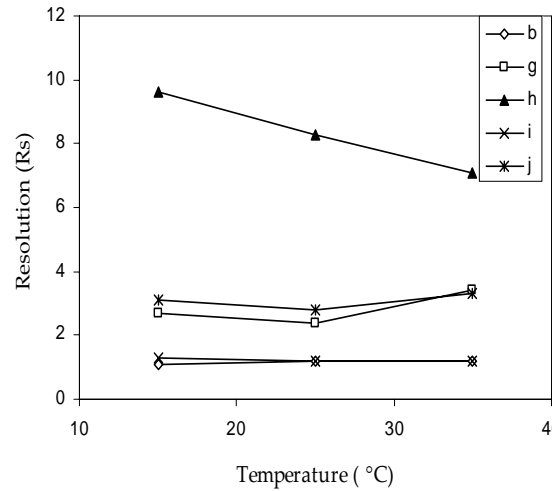
Mobile phase: for k) EtOH/Hex
10/90 v/v; for e), i) and m)
EtOH/Hex 50/50; Flow rate: 0.9
ml/min; Detection: UV@230 nm.

Temperature Effect on Resolution (R_s) Chirobiotic T

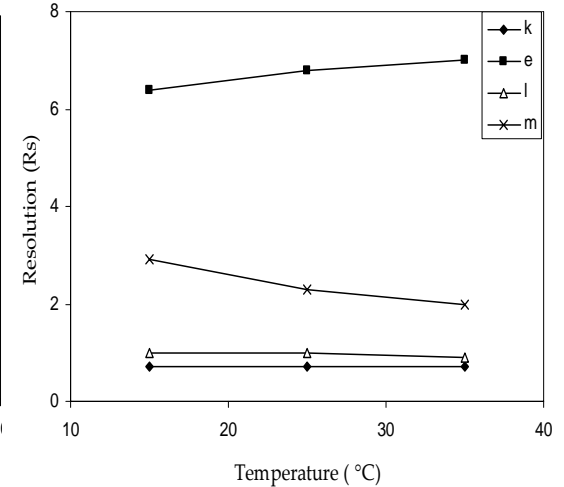
Reversed Phase Mode



Polar Ionic Mode[©]

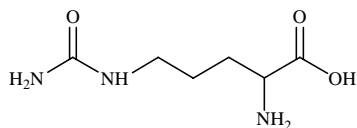


Normal Phase Mode

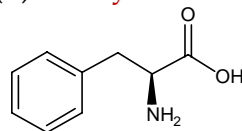


Molecules for the Study of Temperature Effect on Chirobiotic TAG

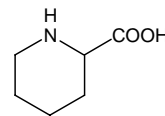
(a) Citrulline



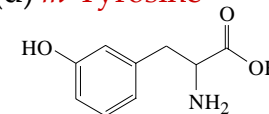
(b) Phenylalanine



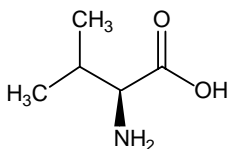
(c) Pilocarpic acid



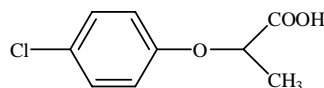
(d) *m*-Tyrosine



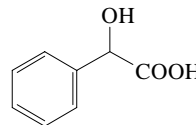
(e) Valine



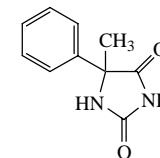
(f) 2-(4-Chlorophenoxy) propionic acid



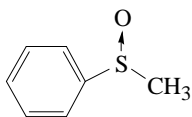
(g) Mandelic acid



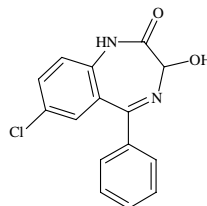
(h) 5-Methyl-5-phenylhydantoin



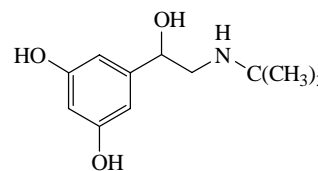
(i) Methylphenylsulfoxide



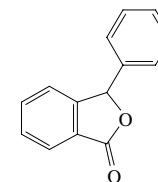
(j) Oxazepam



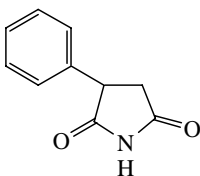
(k) Terbutaline



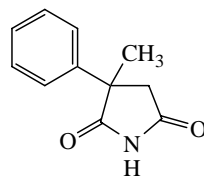
(l) 3-Phenylphthalide



(m) Phensuximide

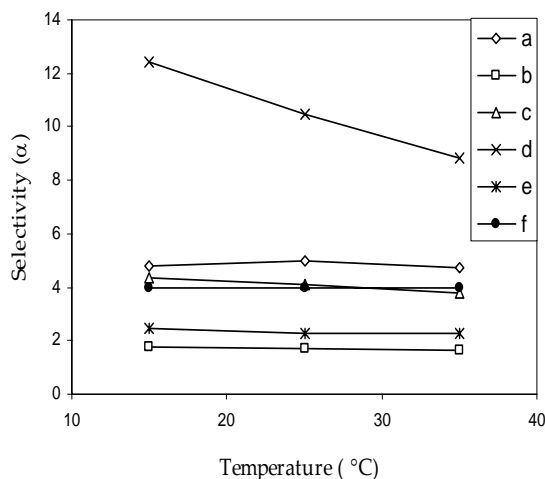


(n) α -Methyl- α -phenylsuccinimide



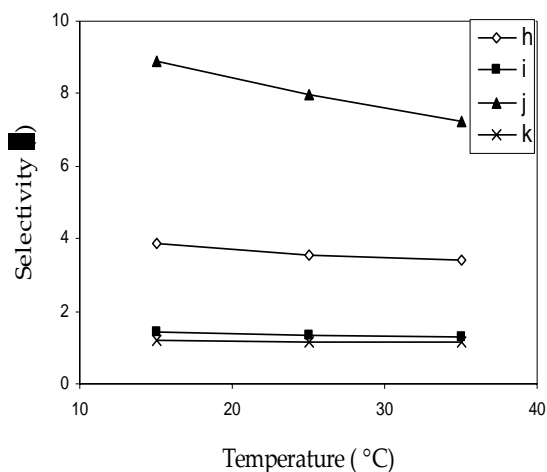
Temperature Effect on Selectivity (α) Chirobiotic TAG

Reversed Phase Mode



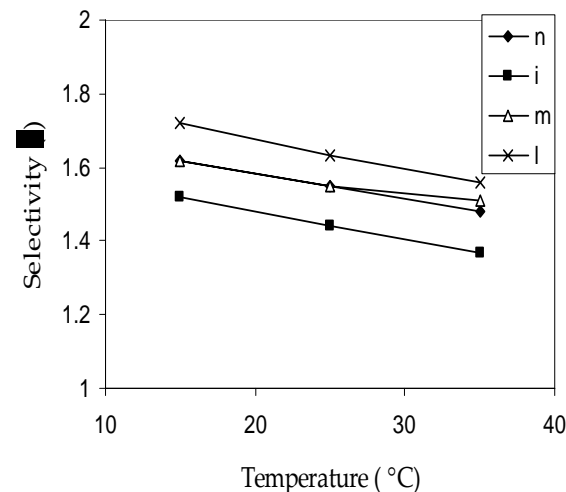
For a), c) and e), Mobile phase: MeOH/0.1% TEAA (pH 4.1) 30/70 v/v; Flow rate: 0.9 ml/min; Detection: light scattering detector.
For b), d) and g), Mobile phase: MeOH/H₂O 60/40 v/v; Flow rate: 1.0 ml/min; Detection: UV@230 nm.
For f), Mobile phase: MeOH/0.1% TEAA (pH 4.1) 20/80 v/v; Flow rate: 0.9 ml/min; Detection: UV@230 nm.

Polar Ionic Mode[®]



Mobile phase: MeOH/HOAc/TEA 100/0.1/0.1 v/v/v; Flow rate: 1.0 ml/min; Detection: UV@230 nm.

Normal Phase Mode

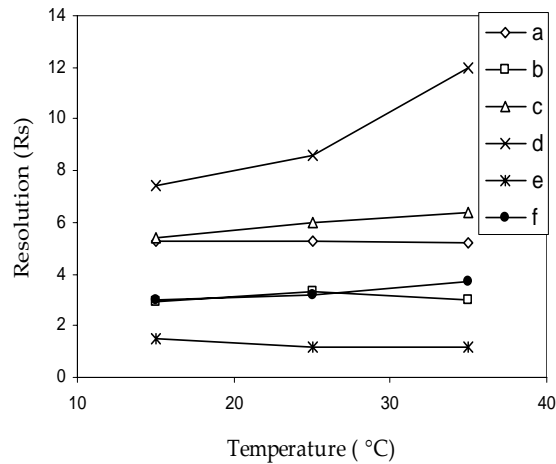


Mobile phase: EtOH/Hex 50/50; Flow rate: 0.9 ml/min; Detection: UV@230 nm.

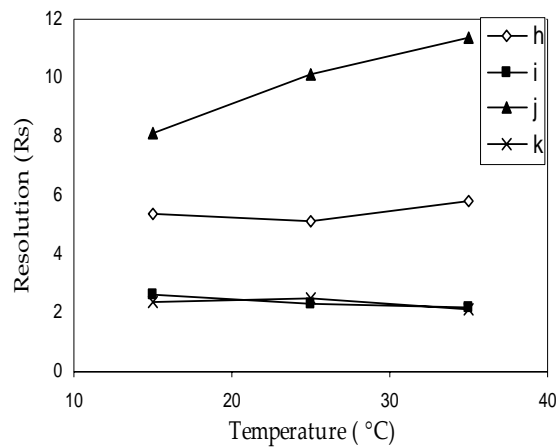
Temperature Effect on Resolution (R_s)

Chirobiotic TAG

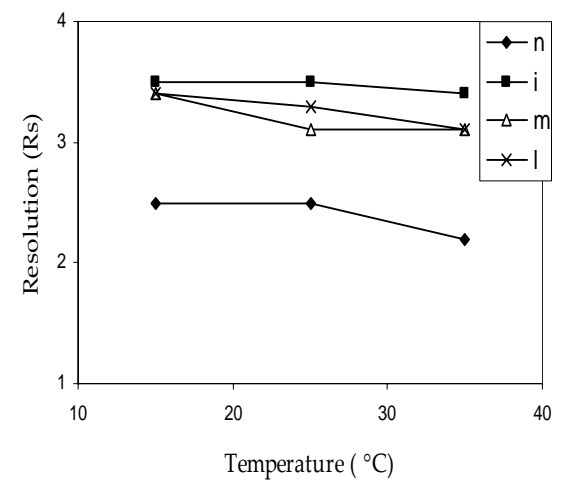
Reversed Phase Mode



Polar Ionic Mode[®]

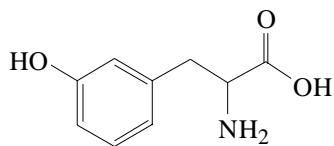


Normal Phase Mode

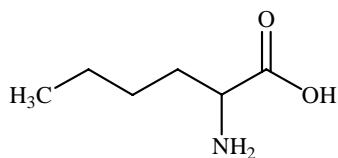


Molecules for the Study of Temperature Effect on Chirobiotic R

(a) *m*-Tyrosine



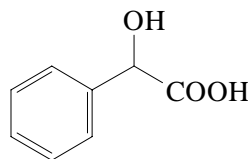
(b) Norleucine



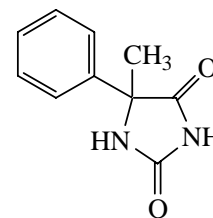
(c) 2-Bromo-3-methylbutyric acid

(d) 3-Indolelactic acid

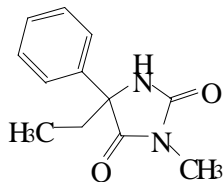
(e) Mandelic acid



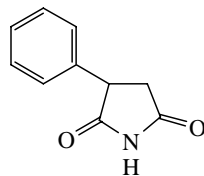
(f) 5-Methyl-5-phenylhydantoin



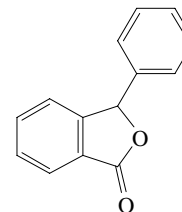
(g) Mephentoin



(h) Phensuximide

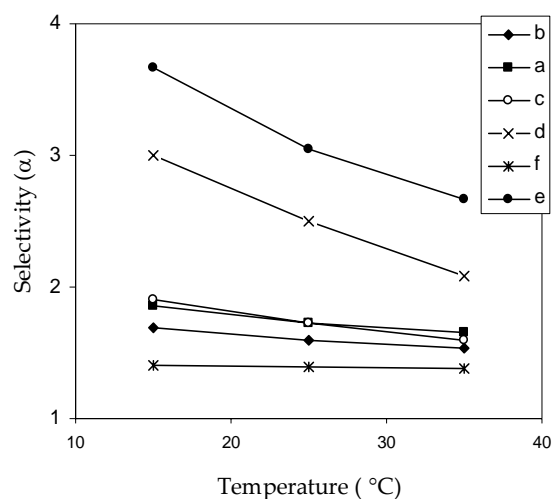


(i) 3-Phenylphthalide



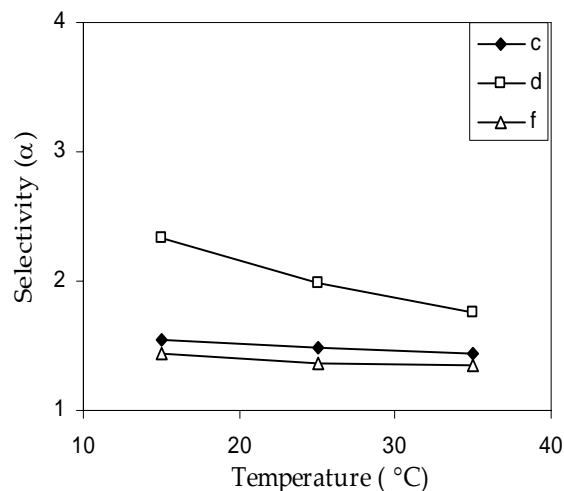
Temperature Effect on Selectivity (α) Chirobiotic R

Reversed Phase Mode



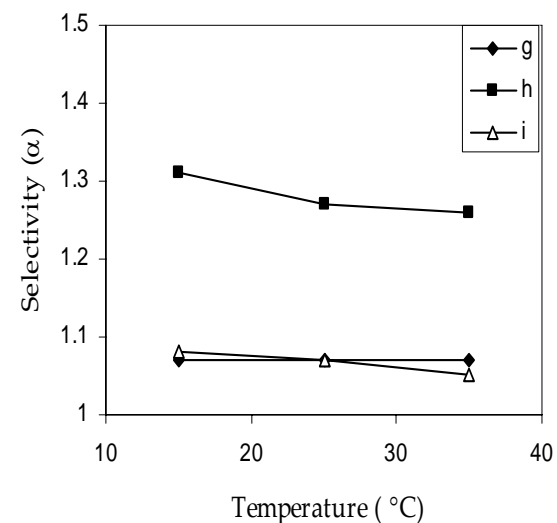
Mobile phase: for a) and b)
MeOH/H₂O 30/70 v/v; for c)-f)
MeOH/0.1% TEAA (pH 4.1) 40/60;
Flow rate: 1.0 ml/min; Detection:
UV@230 nm.

Polar Ionic Mode[®]



Mobile phase: MeOH/HOAc/TEA
100/0.1/0.1 v/v/v; Flow rate: 1.0
ml/min; Detection: UV@230 nm.

Normal Phase Mode

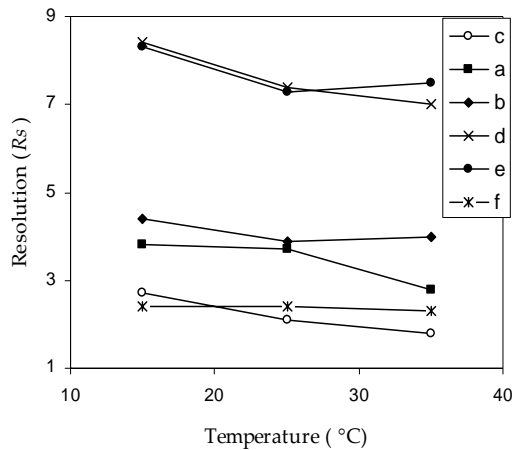


Mobile phase: for g) and i)
EtOH/Hex 20/80 v/v; for h)
EtOH/Hex 50/50; Flow rate: 1.0
ml/min; Detection: UV@230 nm.

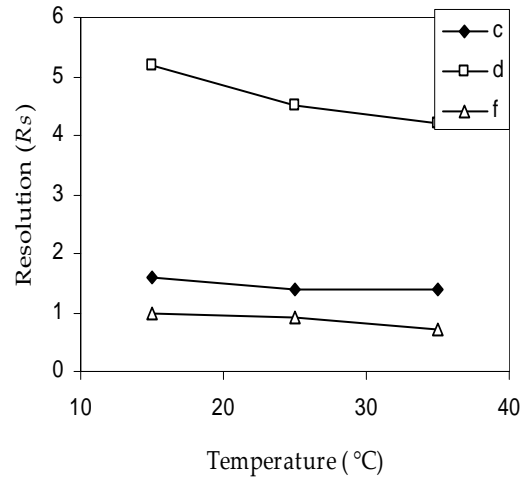
Temperature Effect on Resolution (R_s)

Chirobiotic R

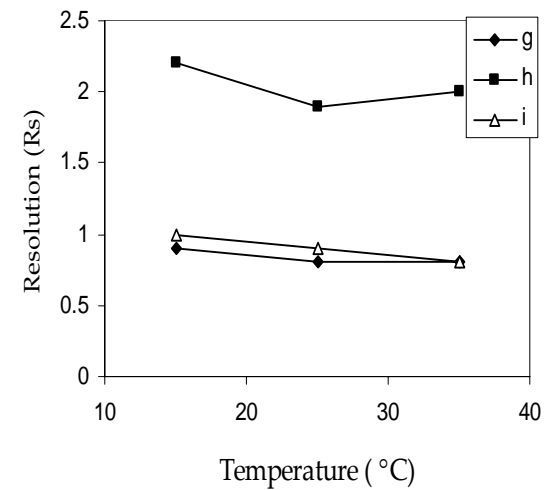
Reversed Phase Mode



Polar Ionic Mode[®]



Normal Phase Mode



Conclusions

- High temperature typically results in high peak efficiency. For most compounds, higher temperature results in shorter retention time. However, opposite retention behavior was observed for β -blockers or molecules with structures similar to β -blockers.
- For molecules subject to on-column racemization or isomerization, separations should be conducted at low column temperatures.
- Lower temperature generally results in better selectivity (α) on all macrocyclic glycopeptide CSPs. The influence of temperature depends on the structures of molecules, the choice of mobile phases and columns. Any structure factors, which improve chiral recognition, will enhance the temperature impact. In most cases, increasing rigidity and bulkiness expect a larger α change when temperature changes. Temperature is more influential in reversed phase mode and polar ionic mode than in normal phase mode.
- Resolution (R_s) does not always follow the same trend of selectivity (α). Especially for underivatized amino acids, higher temperatures usually give better resolutions.
- Temperature-induced reversals of elution order were observed on Chirobiotic V,T, and R, which probably results from the configuration changes of CSPs.