# Temperature Effect on HPLC Retention of PCBs on Porous Graphitic Carbon

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## Key Words

Column liquid chromatography
Porous graphitic carbon
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## Summary

The effect of column temperature on solute retention was investigated for 10 PCBs with different degrees of planarity (degree of ortho position substitution). The variation of retention values (ln $k$) with temperature was measured using two solvents, n-hexane and dichloromethane, as mobile phase: retention decreases as temperature increases. The relationship between ln $k$ values and temperature (1/T) exhibits a good linearity. The $\Delta H^0$ values calculated are in the –2 to –5 kcal mole$^{-1}$ range: they are very similar for compounds with the same degree of ortho-substitution and are significantly higher for non-ortho substituted congeners. High temperature significantly improves separation efficiency (plate number significantly increases): this effect is the dominant factor controlling chromatographic resolution, which improves with increasing temperature. Operating at 40 °C, an efficient separation of planar PCBs was obtained with low solvent consumption.

## Introduction

In HPLC analysis, increasing temperature is one of the most powerful parameters in improving resolution, optimizing selectivity and shortening analysis time [1–10]. The beneficial effects of higher column temperature has led to the development of HPLC at high temperatures (HTHPLC) [11–13], where the column temperature is kept between the solvent's normal boiling point and its critical temperature (usually in the range 20–200 °C). Both theoretical analysis [11] and experimental results [12–13] showed the beneficial effect of high temperatures in improving LC efficiency, speed and performance. Transport properties (diffusivity and viscosity) and chromatographic efficiencies in HTHPLC approach those in SFC [11, 14–15]. However, the practical applications of HTHPLC are still limited: the reasons for this are the lack of systematic studies into the retention behavior of different stationary phases at high temperatures and the complex modifications to conventional HPLC equipment required for operation with superheated liquids [1–13].

The present paper discusses the effect of column temperature on retention behavior in a limited temperature range (20–70 °C) in order to take advantage of the benefits of higher temperature and reduce the complexity of the experimental set-up. The paper is not devoted to the rigorous study of retention process thermodynamics, but to elucidate how temperature controls HPLC separation and how this parameter can be used to optimize analysis conditions.

The HPLC system studied consisted of porous graphitic carbon (PGC) [16] employed to separate polychlorobyphenyls (PCBs) under adsorption chromatography conditions (n-hexane and dichloromethane were used as solvents) [17, 18]. It has been widely reported that solute retention on PGC is determined both by dispersive forces and specific, polar, electronic interactions with the $\pi$ electronic structure of the graphite [5, 16–23]. As a consequence, this stationary phase exhibits an unique selectivity to steric changes in the solute molecule that disturb its electron density and attachment to the graphite surface; thus it is highly selective to positional and stereo isomers [20–25]. Therefore, PGC has been widely employed in analyzing PCBs [24–32]: non-planar congeners (poly-ortho substituted compounds) are separated from the co-planar toxic molecules (non-ortho and mono-ortho substituted PCBs) [33–35], whose concentration determines the toxic impact of an environmental sample [17]. The use of high temperature is proposed as an alternative means for enhancing separation performance in order to overcome the drawbacks of the methods reported to date for PCB analysis: i.e., broad elution profiles [27–32], elution

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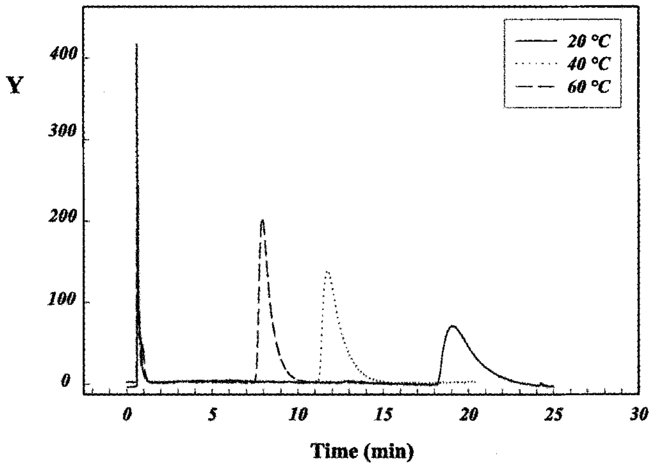

**Figure 1**
Chromatographic peaks of a non-ortho substituted congener (PCB 126) eluted with dichloromethane at different column temperatures: 20 °C, 40 °C and 60 °C.

**Table I.** Molecular structure of the PCB congeners studied.

| Class | PCB n. | Structure |
|---|---|---|
| di-ortho | 52 | 2,2',5,5' |
| | 101 | 2,2',4,5,5' |
| | 153 | 2,2',4,4',5,5' |
| | 180 | 2,2',3,4,4',5,5' |
| mono-ortho | 157 | 2,3,3',4,4',5 |
| | 167 | 2,3',4,4',5,5' |
| non-ortho | 81 | 3,3',4,5 |
| | 77 | 3,3',4,4' |
| | 126 | 3,3',4,4',5 |
| | 169 | 3,3',4,4',5,5' |

back-flushing requirement [25], high consumption of elution solvent [24, 26] and low chromatographic performance for the most highly retained planar PCBs [29–32].

## Experimental

### Standard Solutions

Standard solutions of individual PCBs (10 ppm w/v in isooctane) and solid biphenyl were obtained from Alltech Italia (Milan, Italy).

### HPLC Apparatus

The HPLC apparatus was a Waters 484 (Millipore, Milford, Massachusetts, USA) equipped with a UV detector Waters 501 (Millipore, Milford, Massachusetts, USA). The column used was a 100 x 4.7 mm I. D., 7 $\mu$m particle size Porous Graphitic Carbon (PGC) (Hypercarb, Shandon Scientific, Ltd., UK). The column was placed in a water jacket, the temperature of which was maintained constant to ± 0.5 °C. Eluents were n-hexane and dichloromethane (HPLC grade from J. T. Baker B. V., Deventer, the Netherlands). Both solvents are usually employed in the preliminary steps of the PCB analysis [15] (extraction, clean-up) and are highly volatile, so that they can be easily evaporated before GC analysis. PCB retention data were measured at different temperatures using pure n-hexane and dichloromethane as mobile phase, at a flow rate 1 mL $\text{min}^{-1}$. All measurements were repeated twice; studies on measurement precision showed a 0.2 % reproducibility. Chromatograms were acquired using the Chrom-card DOS Rel 1.30 software (Fisons Instruments, Milan, Italy).

The temperature ranged from 5 to 70 °C for n-hexane and from 5 to 50 °C for dichloromethane, measurements being made at approximately 10 °C intervals. The column was equilibrated at each new temperature for two hours before data collection. In order to maintain the mobile phase in liquid form at a temperature near to or higher than the boiling point of the solvent (69 °C for n-hexane and 40 °C for dichloromethane) a back-pressure was introduced incorporating a suitable restrictor (a capillary 20 mm long and 0.18 mm ID) at the column outlet.

## Results and Discussion

PCB congeners with suitable molecular structures were selected in order to represent 3 classes of substitution – non-ortho, mono-ortho and di-ortho chlorinated compounds – as described by their molecular structures reported in Table I. Also biphenyl was investigated as a reference molecular structure.

With the weaker solvent, hexane, reliable retention values ($\kappa$ in the range 1–20) were measured at different temperatures for the less retained congeners: di-ortho (PCBs 52, 101, 153 and 180), mono-ortho (PCBs 157 and 167) and less retained non-ortho (PCB 81) substituted congeners, other than for biphenyl. With the strong solvent, dichloromethane, retention data were measured for mono- and di-ortho congeners. The experimental data, obtained by eluting PCBs with n-hexane and with dichloromethane, show that with increasing temperature there are two fundamental effects: i) retention decreases giving lower $\kappa$ values; ii) chromatographic peaks exhibit a decreasing width corresponding to higher separation efficiency.

Both these effects are greater for planar, more retained congeners: they are shown in Figure 1, which reports the chromatographic peak of non-ortho substituted PCB 126 obtained at different column temperatures (20, 40 and 60 °C, respectively) eluting with dichloromethane.

### Effect on Retention

Chromatographic retention is an equilibrium process in which a molecule of solute in the mobile phase is transformed into a molecule of solute in the stationary phase [35]. The behavior of the retention factor as a function of temperature is related to the thermodynamics of sorption enthalpy and entropy, determining the sorption of solutes during the chromatographic separation:

$$\ln k = \ln\Psi - \Delta H^0 / RT + \Delta S^0 / R \qquad (1)$$

where ln $\kappa$ is the natural logarithm of the retention factor, $\Delta H^0$ and $\Delta S^0$ are standard enthalpy and entropy change when a solute molecule transfers from the mobile to the stationary phase, $R$ the gas constant and $\Psi$ the phase ratio of the chromatographic system [35–37].

Snyder theory is commonly used to describe retention in liquid-solid chromatographic systems [35]: it was originally developed for oxide adsorbents [35, 38] and subsequently applied to non polar adsorbents such as ODS silica gel [2] and PGC [5, 16]. According to this model, solute retention is the result of a competitive adsorption mechanism of solvent and solute molecules distributed between the bulk liquid and adsorbent layer [35–38]. Therefore, $\Delta H^0$ and $\Delta S^0$ values involved in the retention process are the result of adsorption of a solute molecule (exothermic process) and desorption of corresponding solvent molecules (endothermic process) [2–4, 7, 36].

The effect of temperature on retention can be experimentally evaluated by plotting the log of retention factor against the reciprocal of absolute temperature (van't Hoff plots, reported in Figure 2 for PCBs 157 and 169). When such relationships were calculated from the experimental data, straight lines were obtained across the temperature range examined: the relative standard deviation between experimental and calculated values is lower than 0.2 %, corresponding to the reproducibility of the experimental measurements. Linearity of such plots indicates that:

- $\Delta H^0$ and $\Delta S^0$ values are constant, independent of temperature,
- only one constant binding process is involved in the chromatographic retention,
- no change in the conformational form of solute molecule occurs in the temperature range examined.

In such cases of linear van't Hoff plots, the slope computed by the best fitting straight lines can be used to calculate the enthalpy change $\Delta H^0$ as the solute transfers from the mobile to the stationary phase, while the intercept results in a quantity related to the sum of both the entropy and phase ratio term [2–4, 7]. Tables II and III report these values for PCBs eluted with n-hexane and dichloromethane, respectively.

It can be seen that retention decreases as temperature increases, so that the retention enthalpy is negative, ranging from –1 to –5 kcal mole$^{-1}$. The rather low values indicate that the effect of temperature on retention is moderate: $\Delta H^0$ = –4 kcal mole$^{-1}$ results in a 2-fold decrease in $\kappa$ for a 30 °C temperature increase. The values obtained are comparable to values obtained in RP systems [7], significantly lower than those in NP systems where values less negative than –20 kcal mole$^{-1}$ were computed [36]. This result can be explained by considering that retention on graphitic columns is controlled by weaker forces than those involved in retention on oxide adsorbents. In fact, retention on PGC is determined by London-dispersion interactions (stronger with those planar molecules which can accommodate themselves better on the flat surface of PGC) and polar, electronic interactions – in particular intramolecular interactions of electron pair donor-acceptor and dipole-induced dipole type with the extended $\pi$-electron cloud present on the graphite surface [5, 16, 19–22]. On oxide adsorbents retention is dominated by the possibility of the solute molecule forming hydrogen bonds with –OH groups in the adsorbents, resulting in high enthalpy changes [36].

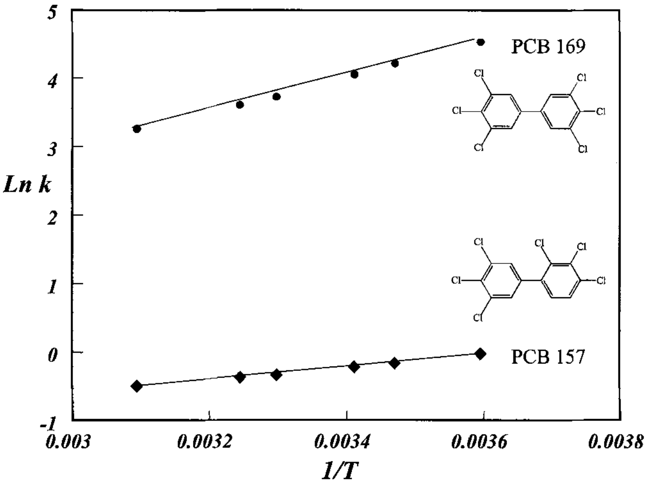

**Figure 2**
van't Hoff plots for a non-ortho substituted (PCB 126) and a mono-ortho substituted congener (PCB 157) eluted with dichloromethane.

Intercept values computed from Eq.(1) are related to entropy changes in the retention process, since the phase ratio term should be constant, all PCBs being eluted on the same stationary phase. For all the PCBs studied very similar values (ranging from –2 to –5) were computed with large associated errors so that nothing could be concluded about the entropy change in retention. It can be only noted that the negative values of $\Delta S^0$ support the concept that adsorption, and consequent loss of freedom for the solute molecule, is the physicochemical process underlying retention on PGC [7, 36].

Specific effects of solute and solvent on retention can be singled out separately.

### *Solvent Effect*

The effect of the solvent can be studied by comparing the retention data of biphenyl eluted with the two solvents. A more negative $\Delta H^0$ value (–3.5 kcal mole$^{-1}$) is obtained with n-hexane than with dichloromethane (–1.38 kcal mole$^{-1}$). This result can be explained in terms of the different strength of the two solvents: dichloromethane, is more strongly adsorbed on the sta-

**Table II.** Intercept (A) and slope values ($\Delta H^0/R$, from which $\Delta H^0$ is derived) of relationship *ln k* vs. 1/T (Eq. (1)): elution with n-hexane (flow rate 1 mL $min^{-1}$).

| Congener | A$^a$ | $\Delta H^0/R^a$ | $r^2$ | $\Delta H^{0a}$ |
|---|---|---|---|---|
| | | | | Kcal mole$^{-1}$ |
| Biphenyl | –5.4 ± 0.5 | –1750 ± 160 | 0.963 | –3.5 ± 0.3 |
| 52 | –4.3 ± 0.3 | –1010 ± 100 | 0.974 | –2.0 ± 0.2 |
| 101 | –4.6 ± 0.6 | –1090 ± 180 | 0.930 | –2.2 ± 0.4 |
| 153 | –3.7 ± 0.2 | –761 ± 73 | 0.972 | –1.4 ± 0.1 |
| 180 | –3.2 ± 0.2 | –732 ± 65 | 0.982 | –1.4 ± 0.1 |
| 157 | –5.0 ± 0.6 | –1840 ± 190 | 0.956 | –3.7 ± 0.4 |
| 167 | –2.9 ± 0.3 | –972 ± 91 | 0.962 | –1.9 ± 0.2 |
| 81 | –2 ± 1 | –1810 ± 370 | 0.933 | –3.6 ± 0.7 |

[a]Errors are expressed as confidence intervals at P = 95 %.

**Table III.** Intercept (A) and slope values ($\Delta H^0/R$, from which $\Delta H^0$ is derived) of relationship *ln k* vs. 1/T (Eq. (1)): elution with dichloromethane (flow rate 2 mL $min^{-1}$).

| Congener | A$^a$ | $-\Delta H^0/R^a$ | $r^2$ | $\Delta H^{0a}$ |
|---|---|---|---|---|
| | | | | Kcal mole$^{-1}$ |
| Biphenyl | –3.1 ± 0.6 | –695 ± 17 | 0.999 | –1.38 ± 0.03 |
| 157 | –3.4 ± 0.1 | –927 ± 30 | 0.996 | –1.84 ± 0.06 |
| 167 | –3.7 ± 0.1 | –996 ± 52 | 0.990 | –1.9 ± 0.1 |
| 81 | –4.4 ± 0.1 | –2053 ± 31 | 0.999 | –4.08 ± 0.06 |
| 77 | –5.1 ± 0.2 | –2302 ± 75 | 0.997 | –4.6 ± 0.1 |
| 126 | –4.7 ± 0.1 | –2346 ± 41 | 0.999 | –4.66 ± 0.08 |
| 169 | –4.6 ± 0.4 | –2530 ± 110 | 0.996 | –5.1 ± 0.2 |

[a] Errors are expressed as confidence intervals at P = 95 %.

tionary phase and competes more efficiently for adsorption sites than does the weaker n-hexane [35, 36, 38]. The solvent desorption process is more endothermic with dichloromethane and mostly compensates for the exothermic solute adsorption process, resulting in less negative retention enthalpy. Analogously, for all the PCBs investigated, the $\Delta H^0$ values obtained with n-hexane are more negative than those obtained with dichloromethane.

### *Solute Effect*

Specific effects of PCB molecular structure on $\Delta H^0$ can be singled out; they are independent of the elution solvent (compare Table II vs. Table III):

– ortho-substitution in the PCB molecule: $\Delta H^0$ values are mainly controlled by ortho-substitution degree of PCB molecules. They are very similar for congeners belonging to the same ortho-substitution class and increase with a decrease in the ortho position substitution degree, according the following order:

di-ortho > mono-ortho ≅ biphenyl > non-ortho

– number of chlorine atoms present in the PCB molecule: within the same ortho-substitution class, $\Delta H^0$ values increase with an increase in the number of chlorine substituents:

4 Cl < 5 Cl < 6 Cl < 7 Cl

– para-substitution in the PCB molecule: $\Delta H^0$ values increase for PCBs with chlorine atoms in the para-position as compared with PCBs with substituents in the meta-position, e. g., PCB 77 vs. PCB 81.

This order corresponds to the retention strength of PCB molecules on PGC stationary phase which is controlled by the planarity of the molecule (i. e., degree of ortho-substitution) and its electronegativity (i. e., number of chlorine atoms present on the biphenyl ring) [16, 19–22].

The effect of ortho-substitution on the value of $\Delta H^0$ is shown graphically in Figure 2, where van't Hoff plots are reported for a non-ortho substituted (PCB 169) and a mono-ortho substituted congener (PCB 157) eluted with dichloromethane: the straight lines obtained diverge at higher *1/T* values, since the plot of the planar PCB exhibits a slope (corresponding to $\Delta H^0$ = –5.1 kcal mole$^{-1}$) higher than that of the mono-ortho PCB 157 ($\Delta H^0 = -1.84$ kcal mole$^{-1}$). This difference in $\Delta H^0$ value determines the effect of temperature on selectivity $\alpha$: for congeners belonging to the same ortho-substitution class $\Delta H^0$ values are very similar (Tables II and III) and therefore selectivity $\alpha$ is more or less independent of temperature. For PCBs belonging to different classes, when van't Hoff plots show a divergent pattern at high *1/T* values (Figure 2 for the pair PCB 169/PCB 157), $\alpha$ values decrease slightly at higher temperatures.

## Effect on Efficiency

The effect of temperature on chromatographic efficiency was evaluated by computing plate number values N for peaks of non-ortho substituted congeners (PCBs 77, 81, 126 and 169) eluted with dichloromethane at different column temperatures (20, 40 and 60 °C, respectively). The results obtained (Table IV) show that separation efficiency significantly improves at higher temperatures [35–37]: a mean increase of 15 % is achieved in plate number when the temperature increases by 20 °C. This effect is related to the decrease in peak width, as shown in Figure 1 (chromatographic peak of PCB 126 eluted with dichloromethane at 20, 40 and 60 °C, respectively). This result is ascribed to the strong influence of temperature on transport and kinetic properties of the chromatographic system. With increasing temperature, mobile phase viscosity decreases and diffusion coefficients, which control mass transfer kinetics, increase [35–37]. The result is that optimum flow rate at the minimum plate height is higher and the slope of the van Deemter curve at high flow rate is lower, so that it is possible to operate at higher flow rate without sacrificing efficiency [2, 11, 12].

## Effect on Resolution

The effect of temperature on chromatographic separation was evaluated by computing chromatographic resolution $R_S$ between two adjacent peaks. As examples, $R_S$ values were calculated at various temperatures (20, 40 and 60 °C) for two pairs of congeners belonging to the same class of non-ortho substituted compounds: PCBs 126–77 and 169–126. The computed results, in Table V. Show beneficial effects in separations can be obtained by operating at high temperatures; mean $R_S$ values increase by more than + 15 % when the temperature changes from 20 °C to 60 °C.

It must be noted that, within the temperature range studied, selectivity values, $\alpha$, for pairs of congeners are fairly constant (see Table V). It can, therefore, be inferred that the parameter determining the enhanced separation resolution is the chromatographic efficiency, which is significantly improved by operating at higher temperatures.

These results were verified on a test mixture containing 10 standard PCBs. An example of a separation was obtained eluting with dichloromethane (flow rate 2 mL min$^{-1}$) operating at 40 °C. Under these conditions (chromatogram shown in Figure 3) non-ortho substituted congeners (PCBs 81, 77, 126 and 169) are efficiently eluted giving sharp and well-separated peaks at low retention times (6.5, 8.0, 11 and 22 min, respectively) consuming a solvent volume lower than 50 ml. A good separation, corresponding to an $\alpha$ value of 1.3, is achieved for the critical pair of PCBs 77 and 81 (5th and 6th peaks).

However, under such conditions, di-ortho PCBs (no. 52, 101, 153 and 180) are nearly un retained and elute closely to mono-ortho PCBs (isomers 167 and 157, at 2.7 and 2.8 min., respectively). This drawback, due to the high strength of the eluent dichloromethane, can be overcome by searching for binary gradient elution conditions: this topic is beyond the aim of the present work and will be discussed in another paper.

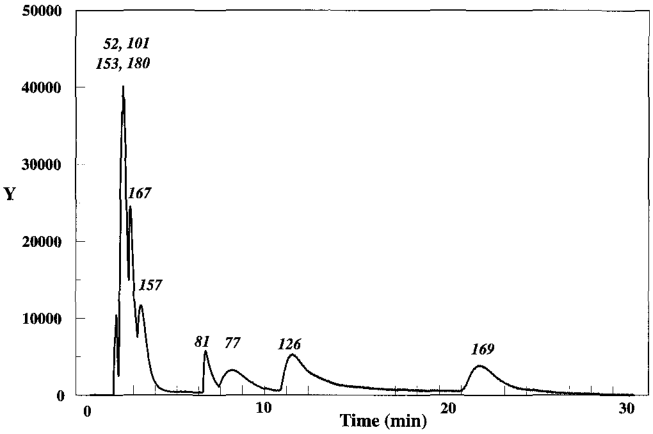

**Figure 3**
Chromatographic separation of 10 standard PCBs obtained operating at 40 °C.

**Table IV.** Plate number, *N* (± standard deviation values) computed for non-ortho PCBs eluted with dichloromethane (2 mL min$^{-1}$) at different temperatures.

| Temperature °C | *N* | *N* | *N* | *N* |
|---|---|---|---|---|
| | PCB 77 | PCB 81 | PCB 126 | PCB 169 |
| 20 °C | 96 (± 2) | 276 (± 8) | 683 (± 17) | 978 (± 24) |
| 40 °C | 190 (± 4) | 456 (± 11) | 786 (± 19) | 1046 (± 26) |
| 60 °C | 279 (± 7) | 509 (± 13) | 804 (± 20) | 1155 (± 29) |

**Table V.** Resolution $R_s$ and selectivity $\alpha$ values (± standard deviation values) computed at various temperatures (20, 40 and 60 °C) for pairs of congeners eluted with dichloromethane (flow rate 2 mL min$^{-1}$)

| Temperature °C | *Rs* 126/77 | $\alpha$ 126/77 | *Rs* 169/126 | $\alpha$ 169/126 |
|---|---|---|---|---|
| 20 °C | 1.04<br>(± 0.03) | 1.73<br>(± 0.04) | 5.17<br>(± 0.12) | 2.07<br>(± 0.06) |
| 40 °C | 1.03<br>(± 0.02) | 1.75<br>(± 0.05) | 5.46<br>(± 0.13) | 1.97<br>(± 0.05) |
| 60 °C | 1.23<br>(± 0.03) | 1.71<br>(± 0.04) | 6.36<br>(± 0.15) | 1.98<br>(± 0.05) |

# Conclusions

Investigation of retention behavior of PCBs on PGC at elevated temperatures has shown that temperature is a powerful means for controlling retention values, column efficiency and chromatographic resolution. Therefore, this parameter is an operational variable that can be selected to optimize separation of PCBs with different planarity/toxicity.

The results obtained are particularly promising in: i) analysis of multicomponent mixtures. The chromatograms obtained give a number of crowded peaks, the separation of which is critical and requires good column performance; ii) analysis of trace level components, where narrow peaks are required to increase the detection limit; iii) separations in binary gradient elution conditions can further improve benefits obtained by operating at high temperatures in order to obtain an efficient separation of all critical congener pairs.

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