Strategies to compare bonded stationary phases of HPLC for the preparative or semi-preparative separation of unsaturated polycyclic hydrocarbons by groups in products derived from petroleum
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STRATEGIES TO COMPARE BONDED STATIONARY PHASES OF HPLC FOR THE PREPARATIVE OR SEMI-PREPARATIVE SEPARATION OF UNSATURATED POLYCYCLIC HYDROCARBONS BY GROUPS IN PRODUCTS DERIVED FROM PETROLEUM

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Abstract

This contribution summarizes methods to compare bonded stationary phases for the compound-class HPLC separations of unsaturated polycyclic hydrocarbons and polar compounds. These strategies are based on the physical chemistry behavior of the functional group of the bonded stationary phase, behavior of the unsaturated polycyclic hydrocarbons under isocratic and gradient elution, regression analysis, retention index, resolution between adjacent groups and behavior of the bonded stationary phase in real samples.

Keywords: Bonded stationary phases, group separation, unsaturated polycyclic hydrocarbons and polar compounds, comparison among bonded phases, preparative or semi-preparative HPLC

1. Introduction

The unsaturated polycyclic hydrocarbons are abundant in products derived from petroleum and are of particular interest because of their role in various petrochemical processes, as well as their possible carcinogenic and mutagenic properties [1,2,3,4,5]. The knowledge of the concentration of these compounds allows us to minimize hydrogen consumptions in fossil fuel processing. Due to the complexity of products derived from petroleum, it is required to separate the unsaturated polycyclic hydrocarbons by classes or groups before making the characterization of the components by means of instrumental methods of analysis [6]. New preparative or semi-preparative methods in HPLC are oriented to search for bonded stationary phases to replace the typical stationary phases of silica and alumina to make separations by groups [7,8]. The market of instrumental analysis offers a great diversity of bonded stationary phases and, therefore, it is required to develop a strategy or to implement a systematic method that allows us to compare and to select an appropriate bonded stationary phase for the preparative or semi-preparative separation of the unsaturated polycyclic hydrocarbons by groups in products derived from petroleum.

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Comparisons based upon columns of the same size, same solvent, regression analysis for the dependence of the *log k'* and *k'* on the number of carbon atoms of the unsaturated polycyclic hydrocarbon, numbers of pi electrons of the unsaturated polycyclic hydrocarbon, number of rings of the unsaturated polycyclic hydrocarbon and number of pi bonds of the unsaturated polycyclic hydrocarbon under isocratic and gradient elution respectively, retention index, resolution between adjacent groups and behavior of the bonded stationary phase in real samples seem to be convenient strategies to compare and to select a bonded stationary phase for the group separations of unsaturated polycyclic hydrocarbons in products derived from petroleum. We have used these strategies to compare columns with the same solvent and packing. Because the column size and solvents are the same, the retention properties are related to the physical chemistry behavior of the functional group bonded to the stationary phase.

2. HPLC columns and solvents.

The strategies to compare bonded stationary phases for the compound class separation of unsaturated polycyclic hydrocarbons and polar compounds have been explained based on the retention behavior of these compounds on the following columns.

Columns: $-[CH_2]_n-NH_2$ (amino), $-CN$ (ciano), $-[CH_2]_n-CN$ (cianoalkyl), ROR (ether), $R(NH_2)_2$ (diamino) and $-[CH_2]_n-CH(OH)-CH_2(OH)_2$ (diol) (25 cm of length, 4.6 mm I.D and 10 μm particle size for isocratic elution)
$-NO_2$ (nitrophenyl), $-[CH_2]_n-NH_2$ and $–CN$ (15 cm length, 4.6 mm I.D and 5 μm particle size for gradient elution).
Solvent for isocratic elution: hexane or heptane.
Solvent for gradient elution: hexane + dichloromethane.
Microsoft Excel has been used to analyze multiple regression data.

3. Comparison based on the dependence of *log k'* on number of carbon atoms (*C*), number of rings (*A*), pi electrons (e_π) and pi bonds (π) of the unsaturated polycyclic hydrocarbons under isocratic elution with non polar solvents (hexane or heptane).

Benzene, naphthalene, phenanthrene, pyrene, perylene and dibenzo[e,h]pyrene were used to evaluate the dependence of log of capacity factor (*Log k'*) on number of carbon atoms *(C)*, numbers of pi electrons (e_π), number of rings (*A*) and number of pi bonds (π) of these model compounds. In Table 1 we have reported a summary of the parameters of regression that were obtained from these evaluations or first approximation. Low values for the typical error (s_r) and intercept (*b*) and elevated values for r^2 and the slope (*m*) are indications of a good calibration straight line. The slope is taken as the ability of the bonded phase to separate the model compounds according to the molecular parameter used for column calibration. That is to say, the bonded phase with greater slope has the best selectivity (*α*) to separate unsaturated polycyclic hydrocarbons according to the molecular parameter consulted ($m_2/m_1 = Log\ \alpha_2/Log\ \alpha_1$). *r* or r^2 indicates the best straight line or calibration line. High slope

and correlation coefficients values are conditions that favor a smaller uncertainty at the time of interpolating to determine molecular parameters of interest.

Regression parameters for Log *k'* Vs number of rings (*A*)						
Column	-NH$_2$	-CN	*diol	*ROR	-(CH$_2$)$_n$-CN	diamine
Slope (*m*)	0.265	0.207	0.1823	0.0627	0.381	0.254
Intercept (*b*)	-0.758	-0.724	-0.6568	0.2065	-1.013	-0.457
Coef. of correl. (*r*)	0.9898	0.9871	0.9355	0.7779	0.9835	0.9775
Coef. of correl. (*r^2*)	0.9797	0.9743	0.8752	0.6037	0.9672	0.9554
Typical error (s$_r$)	0.0698	0.0612	—	—	0.128	0.0866
Regression parameters for Log *k'* Vs number of carbon atoms (*C*)						
Slope (*m*)	0.07833	0.06115	0.0638	0.0262	0.11283	0.07526
Intercept (*b*)	-1.0128	-0.9243	-1.0311	-0.0075	-1.3841	-0.7086
Coef. of correl. (*r*)	0.9988	0.9980	0.9936	0.9896	0.9954	0.9969
Coef. of correl. (*r^2*)	0.9975	0.9959	0.9872	0.9794	0.9907	0.9938
Typical error (s$_r$)	0.0243	0.0244	0.0454	0.0237	0.0681	0.0323
Regression parameters for Log *k'* Vs number of pi bonds (π)						
Slope (*m*)	0.1567	0.1223	0.1277	0.0524	0.2257	0.1505
Intercept (*b*)	-1.013	-0.9243	-1.0311	-0.0075	-1.3841	0.7086
Coef. of correl. (*r*)	0.9988	0.9980	0.9936	0.9896	0.9954	0.9969
Coef. of correl. (*r^2*)	0.9975	0.9959	0.9872	0.9794	0.9907	0.9938
Typical error (s$_r$)	0.0243	0.0244	0.0454	0.0237	0.0681	0.0323

Table 1. Regression parameters under isocratic elution
*Nine standards were used to build the calibration curve.

It can be seen from Table 1 that the bonded stationary -(CH$_2$)$_n$CN has the best selectivity to separate unsaturated polycyclic hydrocarbons according to their molecular parameters *A, C* and π. The amino bonded phase gives the best straight line in all situations. Notice that the number of electrons pi is equal to the number of carbon atoms of the skeleton of the model compounds and, therefore, separation mechanisms based on these two molecular parameters are possible. The behavior of selected model compounds on a given bonded phase is not necessarily followed by all unsaturated polycyclic hydrocarbons of equal number of rings, but the calibration curve must be made with model compounds which they reflect the best conditions of sensitivity and linearity. From the best calibration curve for a given bonded phase we obtain the behavior of other unsaturated polycyclic hydrocarbons.

Other calibration graphs were obtained by plotting *log k'* of benzene, naphthalene, phenanthrene, pyrene, perylene, dibenzo[e,h]pyrene, chrysene, triphenylene, dibenzo[a,h]anthracene and coronene *Vs C, A*, pi electrons and pi bonds. In this second approximation, the best correlation coefficients were obtained for *Log k' Vs C* and *Log k' Vs pi bonds*. The best correlation coefficients were obtained for the ciano bonded phase (-CN) indicating that this column is the best to predict a linear relations between log *k'* and the consulted molecular parameters. The linearity observed for –CN, -(CH$_2$)$_n$CN and amino could be taken as an indication that the system of pi electrons contributed to the retention mechanism of unsaturated polycyclic hydrocarbons. The bonded stationary phases –(CH$_2$)$_n$CN and amino, have the best sensitivity to separate unsaturated polycyclic hydrocarbons according to the number of rings, number of carbon atoms, number of pi electrons and number of pi bonds. The addition of a greater number of model compounds has made worse the regression

conditions, that is to say, we have obtained smaller values for the slope and the correlation coefficients.

The calculated regression parameters for a column are not always applicable to other columns of the same type, because variations in the capacity factors of the same solutes in similar columns can occur.

The tendency of a column to separate unsaturated polycyclic hydrocarbons by groups and the steric effects imposed by substituent groups on the retention characteristics of the unsaturated polycyclic hydrocarbon are two important aspects to be considered. Columns with greater selectivity to separate unsaturated polycyclic hydrocarbons and their alkyl substituted according to the number of rings are better columns to separate by groups. Group separation can happen if the column has little suitability to separate unsaturated polycyclic hydrocarbons whose differences are the localization, type, size, length and number of substituents present in their structure. In this respect, alumina is less dependent on the alkyl and naphthenic substituents than the bonded stationary phase $R(NH_2)_2$.

4. Comparison based on column resolution under isocratic elution with non-polar solvents (hexane-heptane)

We have defined the resolution between adjacent groups of unsaturated polycyclic hydrocarbons under isocratic elution by means of the following expressions.

$$(1) \quad R = \frac{(LogIr)_2 - (LogIr)_1}{2(\sigma_1 + \sigma_2)}$$
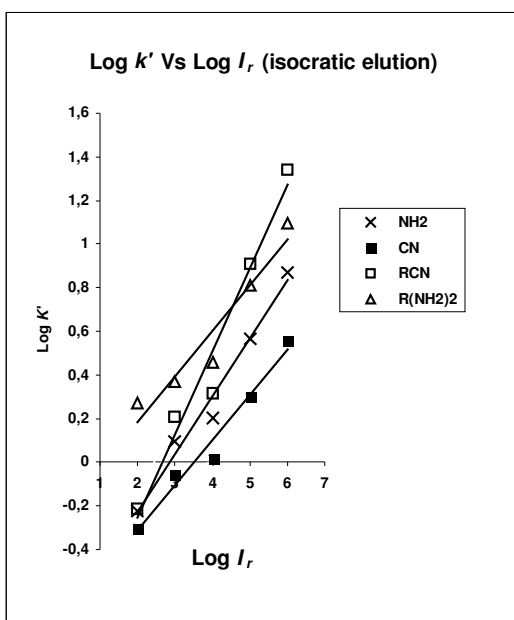

Figure 1. Dependence of Log k' on Log I_r

In this expression $(Log\ Ir)_1$ is the log retention index average for a group and $(Log\ Ir)_2$ is the log retention index average for an adjacent group. σ_1 and σ_2 are the variances of adjacent groups. $2\sigma_1$ and $2\sigma_2$ are the peak groups half width at the base. To calculate the resolutions with these equations, it is required to obtain values of Ir for model compounds of unsaturated polycyclic hydrocarbons. In order to obtain these values it is first necessary to define values of retention index for selected model compounds and to obtain its capacity factors on the column to be investigated. Under isocratic elution $Ir = 10^A$ where A is the rings number of the unsaturated polycyclic hydrocarbon.

The following unsaturated polycyclic hydrocarbons were selected to obtain the calibration curve under isocratic elution. Benzene ($Ir = 10$), naphthalene ($Ir = 100$), phenanthrene ($Ir = 1\ 000$), pyrene ($Ir = 10\ 000$), perylene ($Ir = 100\ 000$) and

dibenzo[e,h]pyrene (Ir = 1 000 000). Under isocratic elution a plot of *log k'* of these compounds Vs Log *Ir* was linear for most of the columns given in Table 1. The behavior of four columns is shown in Figure 1. Some empirical equations will be shown below

$$-NH_2: Log\ k' = -0,758 + 0,2653\ Log\ I_r$$
$$(r^2 = 0,9797)$$
$$-CN: Log\ k' = -0,7238 + 0,2067\ Log\ I_r$$
$$(r^2 = 0,9743)$$
$$RCN: Log\ k' = -1,0126 + 0,381\ Log\ I_r$$
$$(r^2 = 0,9672)$$

Values of *log Ir* for many unsaturated polycyclic hydrocarbons were calculated from these least squares lines. Some values are reported in the following table.

Standard (*A*)	amino	ciano	diol	ether	RCN	$R(NH_2)_2$
naphthalene (2)	2,0 ± 0,36	2,0 ± 0,40	1,9 ± 0,37	2,0 ± 0,59	2,1 ± 0,45	***
phenanthrene (3)	3,2 ± 0,30	3,2 ± 0,34	3,3 ± 0,39	3,4 ± 0,49	3,2 ± 0,38	1,8 ± 0,43
pyrene (4)	3,6 ± 0,29	3,6 ± 0,33	3,7 ± 0,30	3,4 ± 0,49	3,5 ± 0,37	3,3 ± 0,40
chrysene (4)	4,6 ± 0,30	4,5 ± 0,33	4,6 ± 0,30	5,1 ± 0,50	4,3 ± 0,37	3,6 ± 0,38
triphenylene (4)	4,6 ± 0,30	4,5 ± 0,33	4,6 ± 0,30	5,1 ± 0,50	4,3 ± 0,37	4,3 ± 0,37
perylene (5)	5,0 ± 0,31	5,0 ± 0,34	5,2 ± 0,32	5,1 ± 0,50	5,0 ± 0,39	4,4 ± 0,38
dibenzo[a,h]anthracene (5)	5,3 ± 0,32	5,7 ± 0,38	5,6 ± 0,34	6,1 ± 0,60	5,7 ± 0,43	5,0 ± 0,42
dibenzo[e,h]pyrene (6)	6,1± 0,37	6,2 ± 0,42	5,9 ± 0,36	6,2 ± 0,60	6,2 ± 0,47	5,6 ± 0,47

Table 2. Log I_r and interpolation error (isocratic elution)

Nº of rings	-NH$_2$	Si	Al	C$_{18}$	Diamina
1	6	3	5	5	12
2	11	6	6	11	12
3	8	6	5	8	10
4	8	5	4	7	9
5	6	5	—	5	3

Table 3. Nº of model compounds used to calculate resolution

The resolution was calculated from the above equation. The bonded stationary phases RCN and amino gave, under isocratic elution, the largest selectivity to separate unsaturated polycyclic hydrocarbons by the number of rings. The bonded phase amino gave, under isocratic elution, the largest resolution for most of the groups. Five rings unsaturated polycyclic hydrocarbons are difficult to elute from alumina. The nitro bonded phase was not previously considered because unsaturated polycyclic hydrocarbons of more than three rings are difficult to elute from this bonded stationary phase when a non-polar solvent is used.

Nº of rings	-NH$_2$	Si	Al	* C$_{18}$	$R(NH_2)_2$
1 to 2	1,80	0,94	0,48	0,25	1,28
2 to 3	1,37	0,37	0,38	0,14	0,76
3 to 4	0,81	0,24	0,48	0,32	0,48
4 to 5	0,88	0,50	—	1,11	0,55

Table 4. Resolution between groups under isocratic elution

* C$_{18}$ acetonitrile in water

Retention index values to calculate the resolutions of C_{18}, Si and Al were taken from references [12], [13] and [14], respectively

5. Comparison based on the dependence of *k'* on number of carbon atoms (*C*), number of rings (*A*), pi electrons (*e$_\pi$*) and pi bonds (*π*) of the unsaturated polycyclic hydrocarbons under gradient elution with dichloromethane + hexane.

For gradient elution systems, the concept of capacity factors has no theoretical or practical significance due to the fact that it is gradient dependent. Thus, this parameter is not sufficient to compare columns. However, under the same gradient conditions the capacity factor can be used to estimate some retention characteristics of selected bonded stationary phases.

Standard	Molecular parameters				log k'		
Name	*A*	*C*	*π*	*e$_\pi$*	-NO$_2$	-NH$_2$	-CN
benzene	1	6	3	6	-1,100	-0,602	-0,959
naphthalene	2	10	5	10	0,068	0,079	-0,745
anthracene	3	14	7	14	0,412	0,348	-0,244
pyrene	4	16	8	16	0,498	0,386	-0,149
benzo[e]pyrene	5	20	10	2'0	0,677	0,516	0,137
dibenzo[a,l]pentacene	7	30	15	30	0,850	0,730	0,442

Table 5. log *k'* under gradient elution

The regression parameters reported in Table 6, were obtained by plotting the data given in Table 5 as shown partially in Figure 2.

Parameter	-NO$_2$	--NH$_2$	-CN
k' Vs *A*			
Slope (*m*)	1,1615	0,8115	0,4421
Intercept (*b*)	-1,1213	-0,5155	-0,6695
Coef. of correl. (*r*)	0,9968	0,9902	0,9569
Coef. of correl. (*r^2*)	0,9936	0,9805	0,9157
Typical error (s$_r$)	0,2248	0,2762	0,3239
k' Vs *C*			
Slope (*m*)	0,2987	0,2107	0,1162
Intercept (*b*)	-1,6445	-0,9106	-0,9078
Coef. of correl. (*r*)	0,9957	0,9984	0,9770
Coef. of correl. (*r^2*)	0,9914	0,9969	0,9545
Typical error (s$_r$)	0,2611	0,1111	0,2379
k' Vs pi bonds			
Slope (*m*)	0,5974	0,4213	0,2324
Intercept (*b*)	-1,6445	-0,9106	-0,9078
Coef. of correl. (*r*)	0,9957	0,9984	0,9770
Coef. of correl. (*r^2*)	0,9914	0,9969	0,9545
Typical error (s$_r$)	0,2611	0,1111	0,2379

Table 6. Regression parameters under gradient elution.

It can be seen from Table 6 that, under the same gradient elution, the nitro bonded phase has the greatest slope, therefore, this column has the best selectivity to separate unsaturated polycyclic hydrocarbons according to *A*, *C*, π and e$_\pi$, but the behavior of the model compounds used to calibrate the column do not necessarily reflect the

behavior of all unsaturated polycyclic hydrocarbons. The nitro bonded phase also gave the best linear fits for the dependence of *k'* on number of rings. The amino bonded phase showed better linearity than any of the other two polar bonded phases for the dependence of *k'* on the number of carbon atoms, number of pi bonds and number of pi electrons. The regression parameters r^2 and slope, obtained for the ciano bonded phase are inferior to those of amino and –nitro.

The use of a higher number of model compounds changed the regression parameters, but the general tendency observed in a minor number of model compounds stayed the same, that is to say, the nitro bonded phase has the greater selectivity to separate unsaturated polycyclic hydrocarbons according to C, number of pi bonds and number of pi electrons. The amino bonded phase has the best correlation coefficients to separate unsaturated polycyclic hydrocarbons according to number of carbon atoms, number of pi bonds and number of pi electrons. Bonded phases of the type ciano, that showed to have good selectivity to separate unsaturated polycyclic hydrocarbons under isocratic elution, do not behave in a similar manner to that of gradient elution.
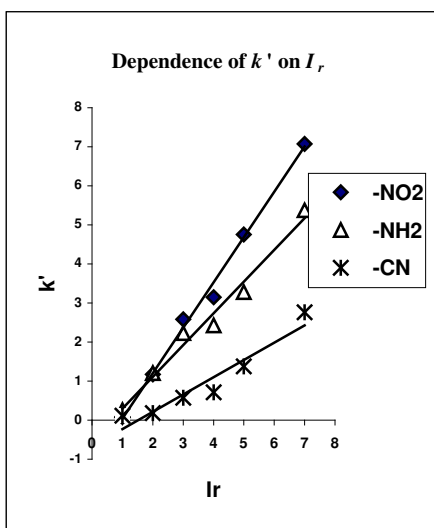

Figure 2. Dependence of *k'* on *Ir* under gradient elution

6. Comparison based on column resolution under gradient elution (hexane + dichloromethane)

The concept of retention index can be extended to a gradient system and we can use it to calculate resolution.

We have defined the resolution between adjacent groups of unsaturated polycyclic hydrocarbons under gradient elution by means of the following expression**.**

$$(2) \qquad R = \frac{(Ir)_2 - (Ir)_1}{2(\sigma_1 + \sigma_2)}$$

.

In this expression $(Ir)_1$ is the retention index average for a group and $(Ir)_2$ is the retention index average for an adjacent group. σ_1 and σ_2 are the variances of adjacent groups. $2\sigma_1$ and $2\sigma_2$ are the peak groups half width at the base. Calibrations curves to obtain I_r values are shown in Figure 2 and its regression parameters in Table 6. In the following table we have reported some retention index values, obtained from these equations. In gradient elution we have defined $I_r = A$ and, therefore, the dependence of *k'* on *Ir* or *A* has the same regression parameters.

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.Compound	Nº of rings	-NO$_2$	-NH$_2$
Name	A	Ir ± σ$_x$	Ir ± σ$_x$
benzene	1	1,03 ± 0,25	0,94 ± 0,41
naphthalene	2	1,97± 0,22	2,11 ± 0,38
anthracene	3	3,19 ± 0,21	3,38 ± 0,37
pyrene	4	3,68 ± 0,21	3,63 ± 0,37
chrysene	4	4,33 ± 0,21	4,39 ± 0,37
naphthacene	4	4,42 ± 0,21	4,18 ± 0,37
triphenylene	4	4,52 ± 0,22	4,35 ± 0,37
perylene	5	5,26 ± 0,22	4,68 ± 0,38
dibenzo[a,h]anthracene	5	5,42 ± 0,23	4,72 ± 0,38
benzo[b]triphenylene	5	5,85 ± 0,23	5,00 ± 0,39
pentacene	5	—	5,35 ± 0,39
benzo[e]pyrene	5	5,06 ± 0,22	5,29 ± 0,37
dibenzo[a,l]pentacene	7	7,06 ± 0,25	7,25 ± 0,37

Table 7. Retention index values (gradient elution)

The ciano bonded phase was not considered to calculate values of retention index, because its regression parameters, under gradient elution, were found to be unsatisfactory. According to Table 7 the amino bonded phase shows the highest interpolation error to 95% limit of confidence. From the same table we also appreciate that both columns predicts the number of rings of an unsaturated polycyclic hydrocarbon within an acceptable error.

In order to calculate resolution we have included a higher number of model compounds and the data was complemented with data reported in the literature. Since the length of the chain bonded to the functional group can affect retention, it is convenient to compare retention caracteristics from colum to column looking for significant differences in all aspects indicated above.

Rings	-NH$_2$	-NO$_2$
1 a 2	1,40	–
2 a 3	1,20	1,50
3 a 4	0,98	0,66
4 a 5	0,81	0,59
5 a 6	–	0,69

Table 8. Resolution (gradient)

The elution of polar compounds on amino and nitro bonded phases with solvents of low to intermediate polarity is almost impossible to carry out. The technique of backflushing based on two columns in series has been used to separate polar compounds from unsaturated polycyclic hydrocarbons [21]. The polar compounds are retained on the stripper column and the second column separates the unsaturated polycyclic hydrocarbons by rings number. In this case the resolution is calculated considering both column.

Comparison among normal bonded phases under gradient elution has indicated that the bonded phases amino and nitro are the best to separate unsaturated polycyclic hydrocarbons of four and five rings and they are also appropriate to investigate molecular parameters. Unsaturated polycyclic hydrocarbons of four and five rings are found frequently in fractions derived from petroleum. In these fractions we can find bezo[a]pyrene and 7,12-dimethilbenzo[a]anthracene which are two of the most powerful carcinogenic known.
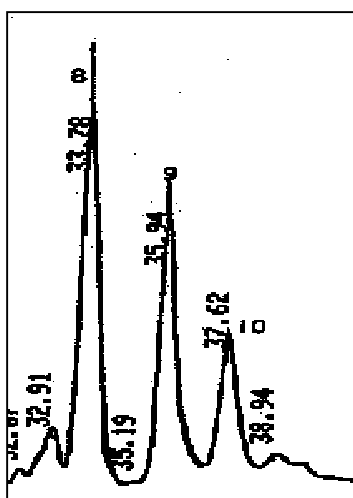
**Figure 3. Reversed phase HPLC
of a diesel extract (UV detector
at 254 nm, acetronitrile + water,
4.6 x 25.0 cm Vydac HPLC column)**

Strategies similar to that described above were employed in selecting a column for the compound class separation of unsaturated polycyclic hydrocarbons in oil shale [3,9], diesel [10,15], light and heavy petroleum distillate [7] and crude oil [16]. These examples have indicated that the amino and nitro bonded stationary phases are appropriated to separate unsaturated polycyclic hydrocarbons according to the ring number and therefore both bonded stationary phases look suitable for the preparative or semi-preparative separation of unsaturated polycyclic hydrocarbons by groups in unfinished oils, lubricating oil base stock as well as other petrochemical products.

The Figure 3 shows a reversed phase HPLC analysis of the five or more rings fraction in a diesel extract after a semipreparative step using normal phase amino column. Peaks 8, 9 and 10 correspond to benzo[b]fluoranthene at 33.78 min, benzo[k]fluoranthene at 35,94 min and benzo[a]pyrene at 37,62 min.
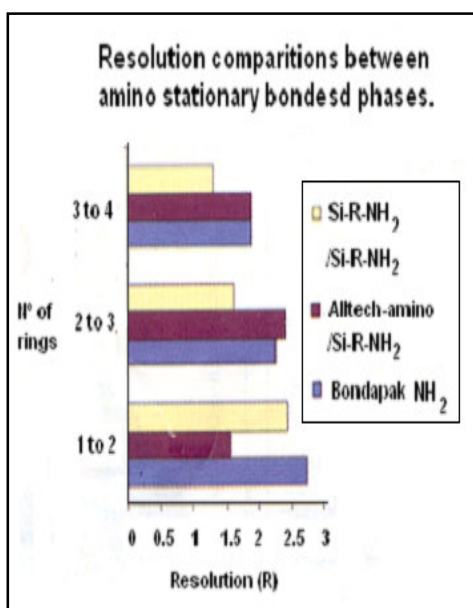

**Figure 4. Resolution comparison of
Amino bonded stationary phases**

There are a variety of amino and nitro bonded phases, but the retention mechanisms explained in this work are similar for all columns bonded to the same characteristic group. The physical properties of a column, like porosity, specific surface area, particle shape and size and pore size greatly determine the eficiency of a packing. The chemical parameters determine the retention mechanisms. The retention mechanisms on bonded phases are determined principally by the properties of the functional group bonded to the stationary phase.

However, it is possible to find differences between the retention of unsaturated polycyclic hydrocarbons on similar bonded stationary phases. In this respect, retention index values are very useful for investigating parameters such as structure, substituent effect and resolution because the retention index of a given compound remain nearly the same even though its retention time may vary several orders of magnitude [17,18]. Figure 4 shows resolution comparisons between amino bonded

stationary phases. Si-R-NH$_2$/Si-R-NH$_2$ and Alltech-amino/Si-R-NH$_2$ means two columns placed in series.

6. Comparison based on the behavior of polar compounds under gradient elution (hexane + dichloromethane)

A wide variety of heteroatom constituents and compounds with attached polar functional groups occur in many products derived from petroleoum [19].When hexane + dichloromethane are used to elute polar compounds on polar bonded phases, polar compounds elute after the unsaturated polycyclic hydrocarbons.

The model polar compounds indole ($pK_b = 17,5$), quinoline ($pK_b = 9,23$), pyridine ($pK_b = 8,94$) e isoquinoline ($pK_b = 8,75$), have an atom of nitrogen with a single lone pair and, therefore, these compounds are hydrogen bond acceptor. Hydrogen protonates the indole ring at carbon two. This protonation disrupts the ring pi-electon system and destroys aromacity, making indole a very weak base. The retention of the weak acid indole on the weakly basic character amino bonded phase can be due to the interaction acid-base of indole with the amino group of the bonded stationary phase or polar-polar interaction betwenn the analyte and the bonded stationary phase.

Nifrogen model compounds	-NH$_2$	-NO$_2$	Oxygen model compounds	-NH$_2$	-NO$_2$
8-methylquinoline	1	1	2-methylbenzenol	1	1
Indole	2	2	4-nonylbenzenol	2	2
2-methylquinoline	3	3	3,4-dimethylbenzenol	3	4
Acridine	4	3	Benzenol	4	3
Quinoline	4	4	naphthalen-1-ol	5	5
Pyridine	5	5	carboxylic acids	6	6
isoquinoline	6	6			

Table 9. Elution order of some representative polar compounds

The presence of an alkyl substituent in indole at the 2 and 3 positions debilitates the indole retention on the bonded stationary phases amino, nitro, and ciano, but these compounds are retained stronger on amino bonded phase than on the other two bonded phases. The presence of the atom of nitrogen in an adjacent position to the fusion side decreases the basicity of the compound and debilitates its retention on the three bonded phases. The quinoline is less basic than the isoquinoline and the first one is less strongly retained on the amino bonded phase than on the nitro bonded phase. Alkyl substituents in quinoline at 2 and 8 positions, affect the formation of hydrogen bridges with the amino group of the bonded phase and the amino bonded phase shows weak retention of 8-methylquinoline and 2-methylquinoline. The size of substituent at the vecinity of the nitrogen atom can also affect the retention of this type of nitrogen compounds. Benzenamine is less basic than pyridine and quinoline and, therefore, it is less retained than the other two nitrogen compounds, that is to say, a mechanism of retention based on the benzenamine basicity explains its weak retention with respect to the pyridine and quinoline. The loss of pi electrons in quinoline to form 1,2,3,4-tetrahydroquinoline reduces the retention of the latest compound on amino, nitro and ciano bonded phases.

In general, it is possible to indicate that the retention order of polar nitrogen compounds on amino bonded phase is largely determined by the basicity of the compound, that is to say, higher basicity involves higher retention.

Basicity order: acridine>isoquinoline>pyridine>quinoline>indole>2-methylindole>3-methylindole

Retention order: isoquinoline>pyridine>acridine≈quinoline>indole>2-methylindole>3-methylindole

The acidity of phenols is a consequence of the interaction of the oxygen of the phenoxyde and the ring. Cyclic substituent groups like phenyl, naphtalen-1-yl, naphtalen-2-yl, etc., bonded to the oxygen are electron attractive groups and its inductive effects also produce acidity. A mechanism of retention based on the acidity of the phenols is consistent with the retention order observed for many phenol compounds on amino bonded phase. The amino bonded phase has weakly basic character and showed strong retention of phenols. Alkyl substitution at a sterically hindered position to the functional group –OH reduces the retention of phenols on the amino bonded phase. For example, 2-methylbenzenol, 2,6-dimethylibenzenol and 2,4-diocthylbenzenol are less retained than benzenol. Nitrogen basic compounds are retained much longer on the nitro bonded phase than on the amino bonded phase. Phenols compounds are retained much longer on the amino bonded phase than on the nitro bonded phase. The nitro group possesses a high dipolar moment. The formal positive charge is localized on the nitrogen atom and it does a great attraction of electrons. As a consequence of this formal charge, nitro bonded phase exerts a strong retention for compounds bases of Lewis.

Ciano is one of the most polar and less basic groups. Due to the poor basicity of ciano group it is also a poor acceptor of hydrogen bonds. Polar-polar interaction is probably the predominant separation mechanism. This column shows preferential retention of basic nitrogen compounds relative to phenols compounds and also shows the effect of alkyl substitution on the retention of some polar compounds.

The nitro bonded stationary phase is not suitable for the separation of weakly polar compounds from unsaturated polycyclic hydrocarbons of 5 to 6 rings.

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