Biphenylpropyl Modified Silica: An Interesting Choice for RP Chromatography

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Alkyl especially octadecyl modified silicas are the workhorses in modern RP chromatography. Their retention mechanism is mainly due to hydrophobic van der Waals interactions. Steric or polar interactions tend to play a minor role. However, some separations are difficult or impossible to achieve with these stationary phases. Therefore, surface modifications with orthogonal selectivity are of great interest. Sorbents for reversed-phase chromatography must have sufficient hydrophobicity and they have to be chemically inert. These restrictions exclude many functionalities. However, aryl ligands as hydrocarbons are hydrophobic and chemically inert [1]. Their ability to undergo π–π interactions affects their selectivity over conventional alkyl phases [2,3]. Silicas modified with phenylpropyl or phenylhexyl silanes show lower hydrophobic interactions and thus shorter retention times compared to octadexyl modified silica. To increase the hydrophobicity of aryl modified silicas biphenylpropyl ligands have been chosen for surface modification. As expected this stationary phase shows enlarged retention times and different selectivities compared to octadecyl phases. They seem to be an interesting alternative to conventional alkyl modified silicas.

Experimental

HPLC grade solvents were used for the preparation of the eluents. In the case of acidic analytes 0.2% trifluoroacetic acid was added to the eluent. The various test compounds were of reagent grade or higher purity and were purchased from several sources.

Depending on the polarity of the analyte, different eluent compositions are chosen, but for each compound only one composition is used on all columns. For comparison of the solvent systems with acetonitrile and methanol the proportion of methanol was chosen so that the eluent systems have a comparable elution force on the reference phase.

The HPLC equipment used in this work was a Vanquish and an Ultimate 3000 UHPLC system (both Thermo Scientific). The dead volume was determined by injection of a solution of uracil in water.

The following stainless-steel columns (125x4 mm) filled with NUCLEODUR® C_{18} Gravity, NUCLEODUR® C_8 Gravity, NUCLEODUR® C_{18} ec, NUCLEODUR® Phenyl-Hexyl, NUCLEODUR® Sphinx RP and NUCLEODUR® π^2 are commercially available (Macherey-Nagel). NUCLEODUR® itself is a totally porous, spherical and high-purity silica gel with an average pore size of 110 Å and a surface area of 340 m²/g.

Results and Discussion

In this work, the behaviour of 86 organic compounds is investigated on different RP phases in comparison to the reference NUCLEODUR® C_{18} Gravity [4]. The presentation of the specific data and detailed HPLC conditions would be beyond the scope of this publication, but they are available from the author. The analytes are organic compounds from various substance classes with a molecular weight below 500 daltons. Only neutral compounds were used, but ionised forms of e.g. anilines or acids could be formed by protonation and deprotonation reactions during chromatography.

The capacity factors $k'(i,c)$ of the individual compounds i determined on the investigated column c are compared with those of the reference phase NUCLEODUR® C_{18} Gravity ($k'(i,ref)$). This column is filled with a sorbent covered with a dense layer of octadecyl ligands. In *Table 1* the used formulas are summarised.

Table 1. Description of the formulas used

$k'_{rel}(i,c)=\frac{k'(i,c)}{k'(i,ref)}$	Relative capacity factor of compound i on column c in comparison to the reference column NUCLEODUR® C_{18} Gravity
$\overline{k'_{rel}(c)}$	Averaged relative capacity factor over all compounds as a measure of the mean interaction strength
$RSD=\frac{\sqrt{\frac{1}{n}\Sigma[k'_{rel}(i,c)-\overline{k'_{rel}(c)}]}}{\overline{k'_{rel}(c)}}$	Relative standard deviation as a measure of the dispersion of the relative capacity factors

Figure 1 shows a plot of the capacity factors of an octadecyl (NUCLEODUR® C_{18} ec) and an octyl (NUCLEODUR® C_8 Gravity) phase with acetonitrile as organic modifier. Using these sorbents, the analytes interact mainly with alkyl groups of different lengths. The similarity of the interactions results in a nearly linear course but with different slopes.

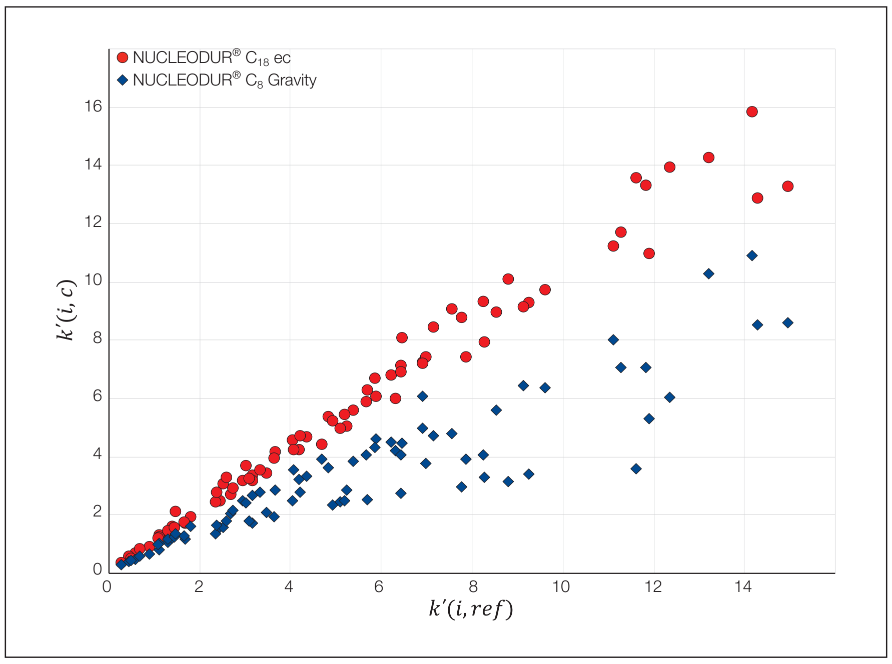
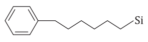
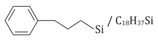
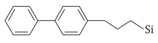


Figure 1. Capacity factors of octadecyl (red) and dense octyl (blue) phase using acetonitrile/water

Figure 2 shows the measured values of two phases modified with aryl groups using acetonitrile/water. The modification of NUCLEODUR® π^2 consists of biphenylpropyl groups, while phenylhexyl ligands are used for the second sorbent.

Figures 3 and 4 show the results of the same stationary phases using the eluent system methanol/water. The scattering of the measured points shows that the biphenylpropyl modification differs markedly from the selectivity of octadecyl and other alkyl modified silicas. To quantify this behaviour, it is necessary to convert the capacity factors *(see Table 1)*. A measure of the differences in the interaction strength of a compound i on column c is the relative capacity factor $k'_{rel}(i,c)$. The average of this value over all compounds chromatographed on a column is a measure of the interaction differences between the investigated sorbent and the reference. The relative standard deviation RSD describes the variation of the relative capacity factors around the mean value. It is a numeric measure of the scattering of points in *Figures 1-4* and describes selectivity differences compared to the reference phase. The calculated values are summarised in *Table 2*.

Table 2. Comparison of interaction strength and dispersion

Stationary phase	Description of the surface modification	Structure	Results acetonitrile		Results methanol	
			$\overline{k'_{rel}(c)}$	RSD	$\overline{k'_{rel}(c)}$	RSD
NUCLEODUR® C_{18} Gravity (reference column)	Dense octadecyl layer	$C_{18}H_{37}Si$	1.00	-	1.00	-
NUCLEODUR® C_{18} ec	Octadecyl layer	$C_{18}H_{37}Si$	1.08	0.09	1.17	0.13
NUCLEODUR® C_8 Gravity	Dense octyl layer	$C_8H_{17}Si$	0.67	0.15	0.55	0.38
NUCLEODUR® Phenyl-Hexyl	Phenylhexyl layer		0.70	0.31	0.75	0.50
NUCLEODUR® Sphinx RP	Dense phenylpropyl/octadecyl layer		0.72	0.29	0.75	0.40
NUCLEODUR® π^2	Biphenylpropyl layer		0.96	0.62	3.10	1.61

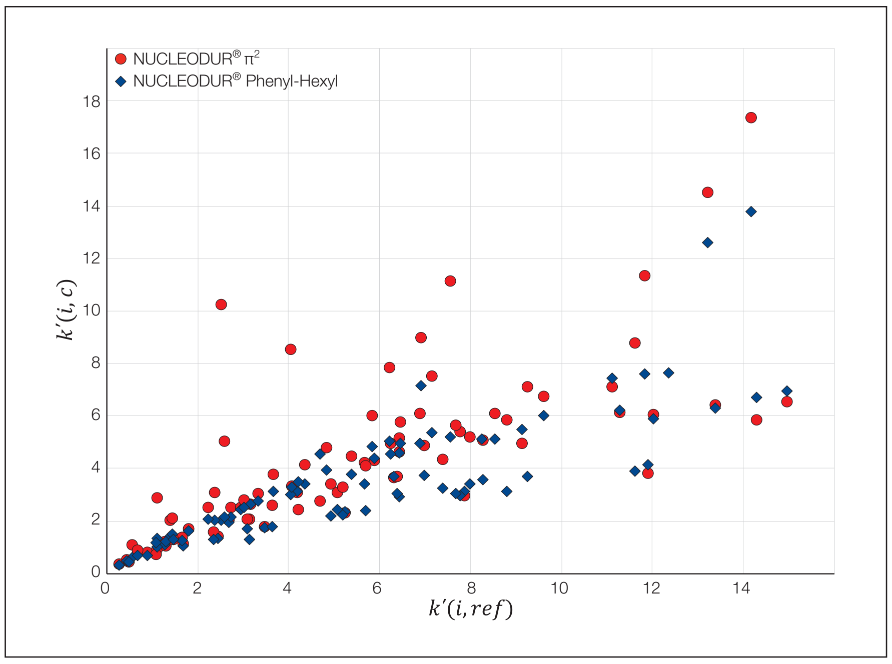


Figure 2. Capacity factors of biphenylpropyl (red) and phenylhexyl (blue) phase using acetonitrile/water

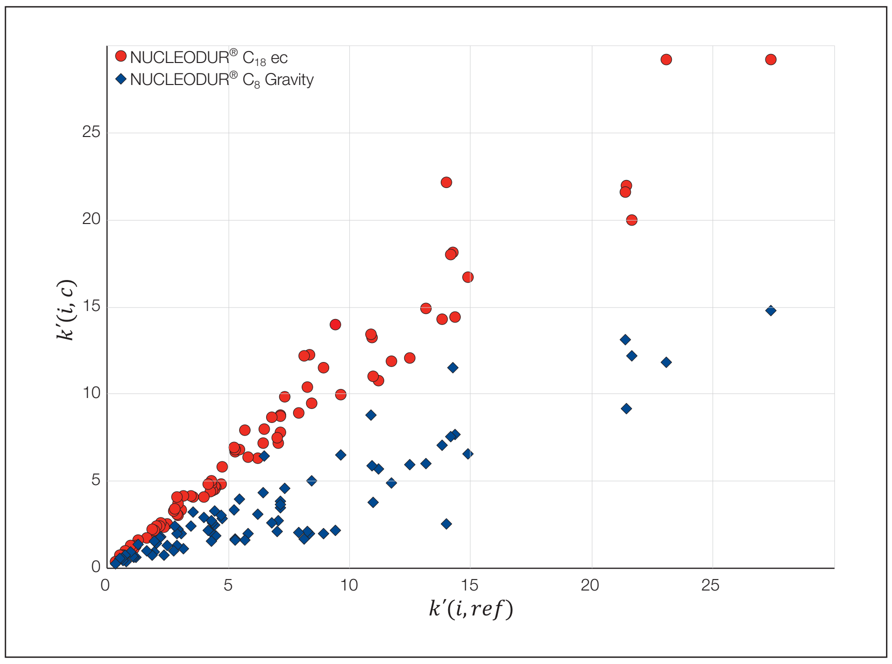


Figure 3. Capacity factors of octadecyl (red) and dense octyl (blue) phase using methanol/water

The averaged relative capacity factor $k'rel(c)$’ shows a mean interaction strength of 108% (1.08) for NUCLEODUR® C_{18} ec. Despite the lower carbon content of the C_{18} ec sorbent, this phase shows a higher mean capacity factor. This indicates that the dense coverage of NUCLEODUR® C_{18} Gravity makes the accessibility of the octadecyl chains difficult. As an octyl phase, C_8 Gravity naturally shows reduced interactions. Using acetonitrile 67% of the interaction strength could be found. The RSD values are relatively small. Since the main separation mechanisms of both alkyl modified phases rely on van der Waals interactions with alkyl groups, these surface modifications differ in their retention times but less in their selectivities.

NUCLEODUR® Phenyl-Hexyl, whose phenylhexyl groups are more hydrophobic due to the longer spacer chain compared to phenylpropyl ligands, reaches an average only 70% (acetonitrile) or 75% (methanol) of the retention factors of the reference column. The relative standard deviation increases to 0.31 (acetonitrile) and 0.50 (methanol), due to the different interactions of the aromatic group. Similar values were also obtained for NUCLEODUR® Sphinx RP. This sorbent is based on less hydrophobic phenylpropyl groups. In order to increase van der Waals interactions, this phase also contains octadecyl groups. The octadecyl ligands present increase hydrophobicity but dilute π–π-interactions.

NUCLEODUR® π² also has aromatic biphenyl groups attached to the silica gel via a propyl spacer. An optimised synthesis method results in a high surface density of the ligands, so that 96% of the interaction strength compared to the reference can be achieved using acetonitrile as organic modifier. The conjugated π-system of the biphenyl group lends to a higher RSD value of 0.62. This is also evident from the greater variability of the relative capacity factors in *Figure 2*.

While there are only slight differences between the averaged relative capacity factors and the RSD values for NUCLEODUR® Phenyl-Hexyl and NUCLEODUR® Sphinx RP when using methanol or acetonitrile, both values for NUCLEODUR® π² increase very markedly when methanol is used. The averaged relative capacity factor increases from 0.96 to 3.10 in combination with a large RSD value of 1.61. For the sorbents investigated an orthogonal selectivity is best achieved by NUCLEODUR® π² using methanol/water.

When changing the application from octadecyl to biphenyl phase, the capacity factors of the analytes can both increase or decrease depending in the structure of the analyte. For steroids, nitroaromatics, sulpha drugs, polynuclear aromatics, often stronger interaction can be observed, while for example, small alkyl aromatics have lower capacity factors.

Figure 5 shows an example of the selectivity differences between octadecyl and biphenyl modifications. A mixture of 3-chloroaniline, ethylparaben, 2-chloro-5-nitroaniline, anisole, butylparaben, chlorobenzene, naphthalene and ethylbenzene are separated using methanol/water. Six of these eight compounds show a reversal of the elution order. Only the elution of chloroaniline and ethylparaben remains unaffected. *Figure 6* shows the influence of the organic modifier on the interactions between analyte and the biphenyl ligands of the silica gel. A steroid mixture consisting of hydrocortisone, prednisone, cortisone acetate, corticosterone, testosterone, norgestrel, desoxycorticosterone and progesterone is separated. Although the elution forces of the mobile phases used on the octadecyl phase are comparable, progesterone elutes on the biphenyl phase after 20 min while it only takes 5 min on the octadecyl phase. It can be observed that many steroidal structures with methanol/water show this behaviour, but the extent of this effect is different and depends on the analyte.

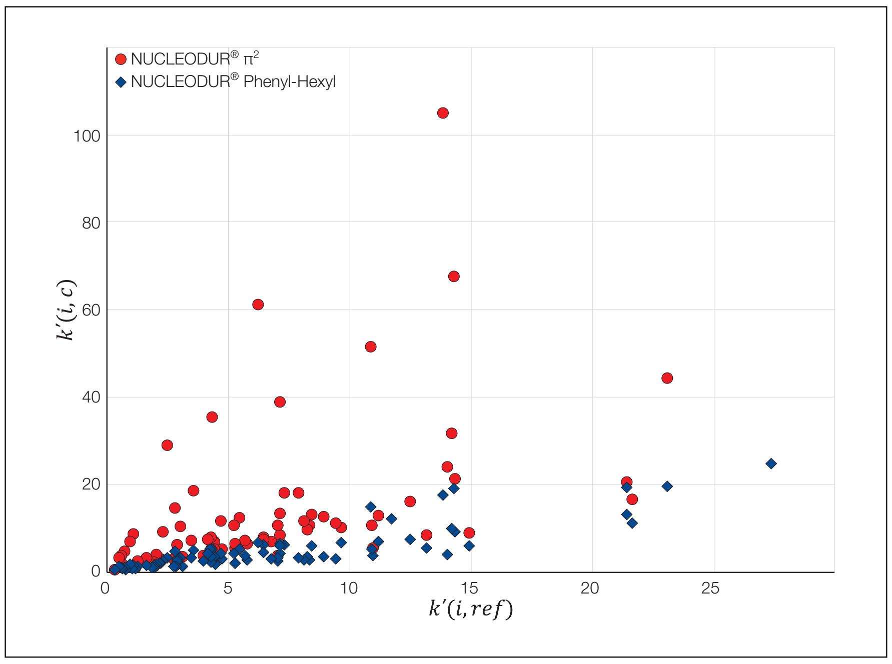


Figure 4. Capacity factors of biphenylpropyl (red) and phenylhexyl (blue) phase using methanol/water

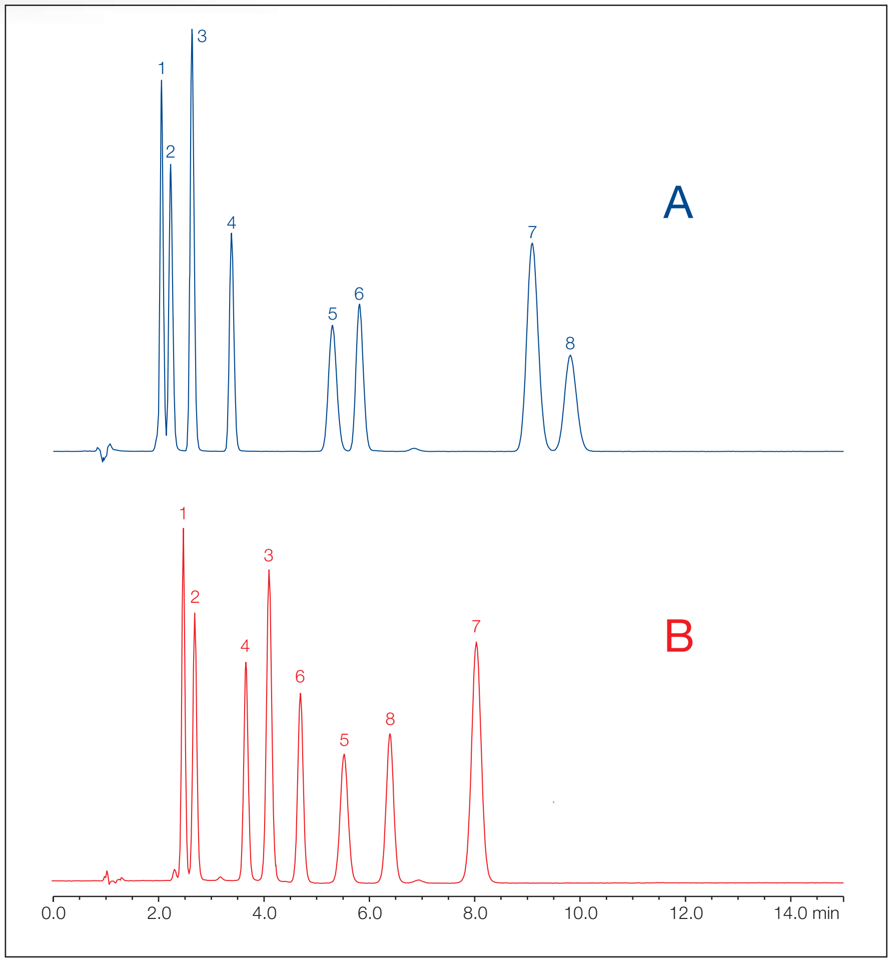


Figure 5. Selectivity differences between octadecyl and biphenylpropyl modifications Chromatogram A EC 125/4 NUCLEODUR® C_{18} Gravity, 5 µm, chromatogram B EC 125/4 NUCLEODUR® π^2, 5 µm, 1 mL/min, 25°C, UV, 260 nm, methanol/water 65:15 (v/v), 1. 3-chloroaniline, 2. ethylparaben, 3. 2-chloro-5-nitroaniline, 4. anisole, 5. butylparaben, 6. chlorobenzene, 7. naphthalene, 8. ethylbenzene

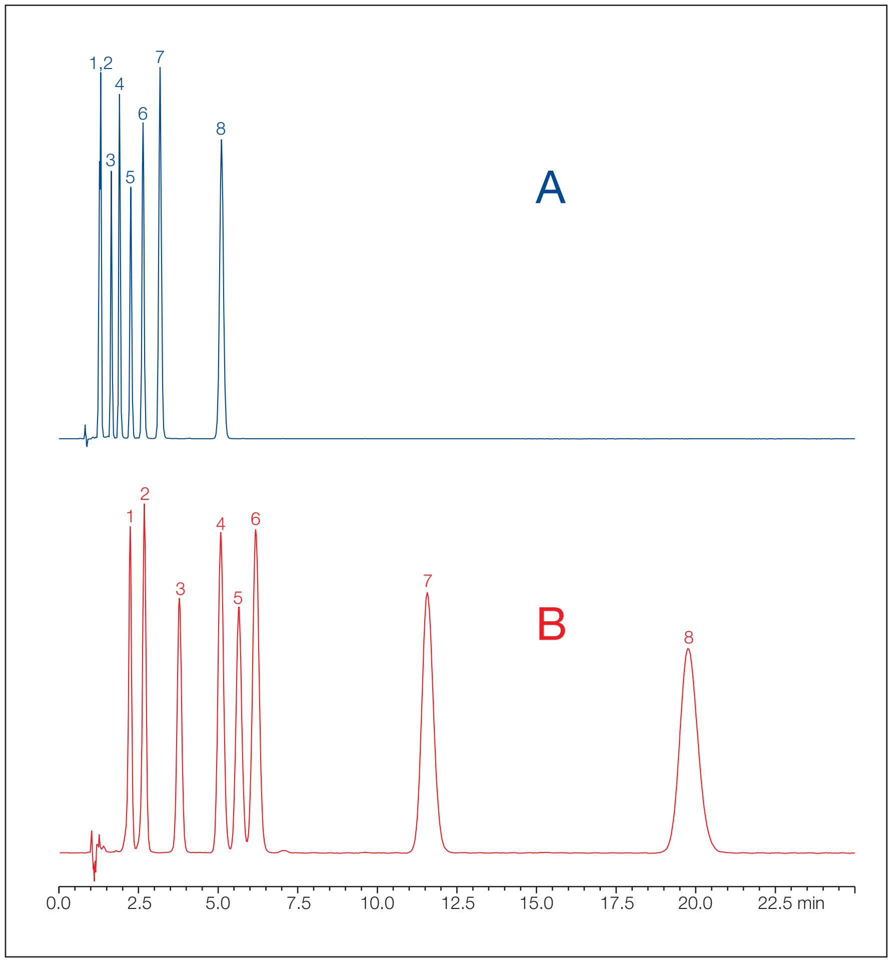


Figure 6. Influence of the organic modifier using biphenylpropyl silica EC 125/4 NUCLEODUR® π^2, 5 µm, 1 mL/min, 25°C, UV, 240 nm, chromatogram A acetonitrile/ water 75:25 (v/v), chromatogram B methanol/water 85:15 (v/v), 1. hydrocortisone, 2. prednisone, 3. cortisone acetate, 4. corticosterone, 5. testosterone, 6. norgestrel, 7. desoxycorticosterone, 8. progesterone

Conclusion

NUCLEODUR® π^2 is a new stationary phase for HPLC separations, characterised by orthogonal selectivity to conventional octadecyl modified silicas. In the eluent system acetonitrile/water, the interaction strength is mostly comparable to RP18 phases. But in the system methanol/ water the interaction strength and with it the retention is significantly superior. Also, the selectivity differences are more pronounced here. It is therefore a very interesting and effective alternative to conventional RP phases, if their selectivity is insufficient.

References

[1] F. Chan, L.S. Yeung, R. LoBrutto, Y.V. Kazakevich J. Chromatogr. A 1069 (2005) 217-224

[2] M.R. Euerby, P. Petersson, W. Campbell, W. Roe J. Chromatogr. A 1154 (2007) 138-151

[3] K. Croes, A. Steffens, D.H.Marchand, L.R.Snyder J. Chromatogr. A 1098 (2005) 123-130

[4] H. Riering, N. Bilmann, G. Cozzupoli, Comparison of various aryl and alkyl modified sorbents in RP chromatography, Poster ISC 2016, Cork (Online available www.mn-net.com/NUCLEODUR (Poster: Comparison of RP sorbents))