

Guide to Choosing and Using Polymer Reversed Phase Columns!

Choosing the best RP Column to use:

Silica is normally used as the packing material for HPLC columns for a number of reasons. It is very strong, allowing it to be used at high back-pressures, and packed at even higher pressures to give a really stable columns with sharp peaks. It is also relatively cheap, so columns cost between £150-£300.

However there are limitations with silica-based columns, which we have all come to accept, but which are a nuisance nevertheless! It dissolves in water, especially at elevated temperature, at higher pH, and in higher buffer concentrations. Hence for many methods, (especially those for basic substances such as amines where we would normally choose to work around pH9-10) we have trade-off between getting a good separation, and not dissolving the column too quickly. This pH limitation can also limit us for column cleaning.

Other problems are that the bonded phase can easily be stripped off by pH less than 2 because the siloxane (Si-O-Si) bond is hydrolysed, and the residual silanol sites (Si-OH groups) on the surface can cause peak tailing.

Various approaches are available to minimise the impact of these limitations, but the most popular solution is to use a column with a polymer-based packing material. For many, this is perceived as an expensive and unknown territory. However since the columns work really nicely and last for absolutely ages, it is actually an easy and cost effective way to solve a lot of problems at once! So here is a guide through the maze.

Polymer columns come in a steel tube, just like silica-based columns, but the gel inside is a rigid polymer matrix rather than silica. There are four main polymers which are used, and they each behave a little differently from silica-based C18.

Polymethacrylate. ([Shodex DE series columns](#))

Polymethacrylate is a polymer that was developed originally for HPLC, although it now has other uses too. It has good temperature, pH and pressure stability, it is compatible with aqueous and organic solvents, it has well defined pore size, and a large surface area!

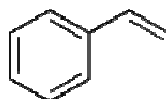
It also forms spherical particles, and hence is relatively easy to pack efficiently in a column. It is a non-polar material, and is used underivatized (*no C18 chain bonded on*) for reversed phase HPLC (RP-HPLC), just as silica is for normal phase columns. Its polarity is virtually the same as a C18 column, but the selectivity (*the position of the peaks relative to each other*) is a little different. When transferring a method from a silica-based C18 column, we recommend using a slightly stronger eluent (*a few % more of the organic solvent*).

These columns can be used with a pH range of 2-12, which gives excellent flexibility for all types of samples. Eluents should be made from Methanol:Water or Acetonitrile:Water but not Tetrahydrofuran (THF). The packing is not dissolved in THF, but it causes the gel to swell, disturbing the packing of the column and causing high back-pressure.

The maximum pressure is 2250psi, and for best efficiency the flow rate should not be over 1.2ml/min for a 4.6mm id column. If a buffer salt is used, the concentration should be kept below 0.5M, although in HPLC we would hardly ever go anywhere near that anyway.

If you are transferring a method from an ODS column, this is a good one to start with! It is very well mannered, you will have no problems with silanols giving peak tailing or the column dissolving, it is packed with an efficiency of at least 70,000N/m so the peaks will be sharp, and there's no bonded phase to strip off. For comparison, a 150 x 4.6mm column costs £466.00 (+VAT), and if you keep your samples free from particles, the column will last for ages!

Styrene/Divinyl Benzene Co-Polymer. ([Shodex DS and RP18 series columns](#))



Styrene (=vinyl benzene) readily forms a polymer because the vinyl groups link together to form a chain (like a washing line with the benzene rings hanging down!). Divinyl benzene has a second vinyl group (meta or para to the first one) and by adding this to the mixture, it forms part of the polymer and allows the polymer chains to form cross-links between them, forming a much stronger and more rigid polymer.

For some industrial applications, very low percentages of DVB are added, but for HPLC much more DVB is used to give a high density of cross-links, and one of the most robust polymer supports available for HPLC. This reaction is well documented and able to be very precisely controlled, allowing the formation of small spherical particles with a very narrow particle size distribution. For reversed phases applications, styrene/divinyl benzene columns are used underivatized.

Columns packed with this material can take over 3000psi, and have a usable pH range of 1-13. Swelling in organic solvents such as THF or chloroform is negligible because of the cross-linking, although it is recommended that the column is not used in totally aqueous eluents. As a guide, it is best to keep a minimum of 5-10% organic solvent with these columns. (For applications requiring 100% water as eluent, use the DE or ODP columns.) The packing is very efficient and efficiencies are guaranteed to exceed 70,000N/m for DS columns and 80,000N/m for RP18, using 3.5µ particles.

These columns are more retentive to hydrophilic samples than a silica-based C18 column, which can be a real advantage. This gives a different selectivity to the DE columns, and as with any selectivity change, this can help in some cases and cause co-elution in others. These columns are recommended to complement the DE series when developing methods, and this is the column of choice when using a method developed using styrene divinyl benzene columns from other manufacturers. For comparison, a 150 x 4.6mm column costs £499.00 (+VAT), and again, they last a very long time!

Polyvinyl alcohol. ([Shodex Asahipak ODP40 and ODP50](#))

Polyvinyl alcohol is a polymer which is unusual in that it is not made by polymerisation of vinyl alcohol. Instead it is made by hydrolysis of polyvinyl acetate, replacing the acetate groups with OH. The surface is much more polar than the other two polymers discussed so far, but because the OH groups fit sterically inside the polymer chain, the external surface is actually not very hydrophilic.

Nevertheless, column packings based upon PVA almost always have a bonded phase attached. In the case of the ODP range, a choice of C₁₈, C₈ and C₄ columns are available, offering a wide range of polarity, C₄ being the most polar. Shodex PVA columns have a wider pore size than the other two.

This means that they can be used with molecules up to a molecular weight of around 200,000, and without the pressure sensitivity that ensues with wide pore silica columns. It does however reduce the surface area a little, so these columns are slightly less efficient than DE or DS columns.

Because the columns have a C₁₈ group bonded on, they have a very similar polarity to a silica based C18 (ODS) column (and similar to a DE column). This makes the transfer of methods from silica ODS columns much easier. They also have a similar selectivity, so the separation should look very similar.

ODP40 is a smaller particle version of ODP50, giving higher efficiency and sharper peaks.

For those wishing to do direct comparisons, the carbon loading is 17%, very similar to most modern type B silica-based C18 columns, and the column can be used with eluents up to pH13. It is recommended that the organic modifier should be Methanol or Acetonitrile but not THF. In general, Acetonitrile gives the sharpest peaks with this column.

For comparison, a 150 x 4.6 column costs £449.00 (+VAT) for ODP50 and £470.00 (+VAT) for ODP40.

[Polyhydroxymethacrylate \(NEW - Shodex ODP2 HP\)](#)

This material has been developed specifically to make silica columns obsolete! It has all the advantages of a polymer material (wide pH range, no silanol interactions, really long column life) but it also is more efficient than any other polymer based column, giving really sharp peaks. It also has excellent retention of hydrophilic materials, and good selectivity. But the most important difference now is the price. This column is available for no more than the best silica columns. For comparison, a **150 x 4.6mm column costs just £285!** This material was only launched in September 2006 and is available now.

[Column Evaluation Program:](#)

If you would like to try one of these columns in your lab, Shodex will send out columns on a sale or return basis. Send an order to us, we will obtain the column from Shodex send it to you to try. You can keep it for up to three weeks before we send you an invoice. If you are not completely happy, you can return the column and owe nothing. Otherwise we then send an invoice as normal.

Alternatively, we have all four of these columns in our lab set up and ready to go. If you would like us to try running a sample for you on each of these four columns, we will do it for free. If we find that a small eluent change would help we will make the change and send you a set of four chromatograms so that you can see what each of these columns could offer you. To arrange this, please call us on 01634-294001, and then send us your sample and method. As soon as we receive it, we'll call you to discuss, and run the separations for you. Once we have the results, we will contact you again to explain what we have done, and answer any question you may have.

[Using Reversed Phase Polymer Columns:](#)

If you are used to silica columns, the most important thing to say first is that it is very important to look up the pressure limit for a polymer column, and set your Max pressure cut-out accordingly. Polymer packings are not so rigid as silica, and can be compressed by really high pressure. Pressure can easily be higher than expected (for example if the eluent mix changes, the column oven is not turned on, or the flow rate is too high.) This can damage the column, and although it will often recover (given a low flow rate overnight) this is not guaranteed and it is a shame to destroy a really good column by accident!

There are several things you can do to minimise the effect of the pressure limitation:

1. First, try using a **lower flow rate**. It is quite normal to operate a 4.6mm id polymer column at 0.6-0.7ml/minute. This offers a 30-40% reduction in back-pressure, and an increase in efficiency! It does make the peaks come out more slowly, but to overcome that it is usual to work with a higher %B in the eluent.
2. For most polymer reversed phase columns, it is necessary to **increase the organic component of the eluent by about 5%**, sometimes a little more. This brings out all the peaks more quickly (although of course the solvent front is unchanged). It has the added benefit that the viscosity of the eluent is reduced, and hence the back-pressure is similarly reduced.
3. Anything which reduces viscosity will reduce back-pressure. So if possible it is a good idea to develop methods to **run at elevated temperature**. Polymer columns should not be run at high temperature, but 35-50°C is fine for any of the above columns. Be careful when turning on the system from cold though, because the back-pressure for the first 15-20 minutes will be higher until the column oven gets up to temperature! If necessary, start with a lower flow rate.
4. **Keep buffer concentrations in check**. The higher the buffer concentration, the higher the back-pressure. You will need at least 0.005M, preferably 0.01M, or the buffer won't be strong enough to act as a buffer. But don't go to 0.1M or above if you don't have to!

The pH range of these columns is really excellent, and a good reason to use them. All will work from pH2-12, some from 1-13. This offers several advantages. Firstly when analysing basic materials (usually containing an NH₂ functional group), it is desirable to work at high pH to prevent ionisation of the amino group, because that gives broad and tailing peaks. There are lots of compromises available using modified silica columns, all of which have some limitations, but the fact remains that to one extent or another, silica dissolves over pH7, and the rate of dissolution increases with pH, temperature, water content of the eluent, and buffer concentration! All of these problems just go away with a polymer RP column!

Pore size is an important point, because with these columns a wide range of pore sizes are offered. First note that 90% of the surface area (and hence 90% of the active sites on the packing material where retention occurs) are inside the pores. It is therefore absolutely essential that the molecules to be analysed are not too big to enter the pores. This applies to all columns, both silica and polymer based.

As a guide:

<i>Pore Size (A)</i>	<i>Approx Molecular Size Limit</i>
40	1000
80	2000
120	5000
250	200,000
300	250,000
430	500,000

There can be advantages of both large and small pore sizes. The largest offered in the columns above is the RP18-415, which has 430A pores, and is recommended for the analysis of proteins. ODP40 and ODP50 columns have 250A pores, so can be used for proteins and peptides as well as smaller molecules. The DE, DS and RP18-413 columns have a pore size of 100A, which is a good general choice, and the ODP2-HP has 40A pores.

It is worth noting that the ODP2-HP column is an excellent general choice, provided that the molecules to be analysed have a MWt less than 1000. This is because it has a high surface area, so has a good loading capacity, and any impurity which is a large molecule (proteins etc or large industrial polymers) will have a very much

reduced attraction to the column because it is excluded from the pores. For example, when analysing foods or milk, where there is a possibility of extracting protein with the material to be analysed, this is a big advantage. Because 90% of the material is unable to bind and any retained material is outside the pores, the column is also much easier and quicker to clean! And with the pH range available, it is considerably easier to remove such molecules than with a silica based column.

Finally, selectivity. This is the main area where these columns offer advantages. Each polymer packing material is non-polar on its own, so apart from the PVA-based materials with a C₁₈ chain bonded on, each is used underivatized to achieve a reversed phase packing. It is therefore no surprise to learn that they are each quite different from each other, and different from silica-based C18. The overall polarity is designed to be similar, so methods do not need major changes. But the peak separation is definitely different. This can be an advantage or a disadvantage, depending on how good a separation you have already. But it makes these columns invaluable when trying to find a column which will offer alternative selectivity for difficult samples, because they offer a real change, not just a slight difference.

One column which is worthy of special note is the ODP2-HP. This is very retentive for hydrophilic materials, and can separate species which would just wash straight off a normal ODS column. For example, we tried uracil and 5-fluorouracil and found that both were retained and we got a good separation.

Should you wish us to try a sample through all four types of columns for you, we will be pleased to do so. To arrange this, call Dr Stuart Jones on 01634-294001. Alternatively you can email stuart@laserchrom.co.uk. You will need to send a sample, ready for injection, and details of any method you currently have. This offer does not include method development for unknown materials, although this can be offered for an agreed cost.