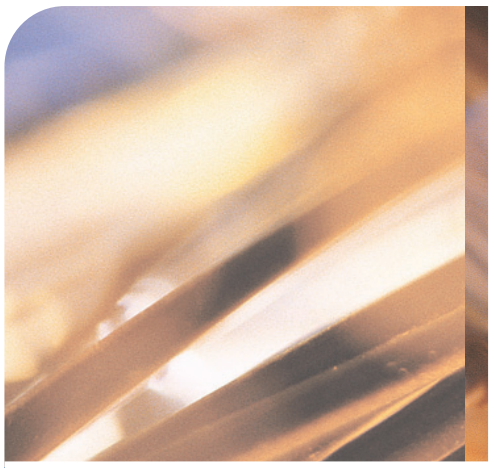


Chromatography Focus

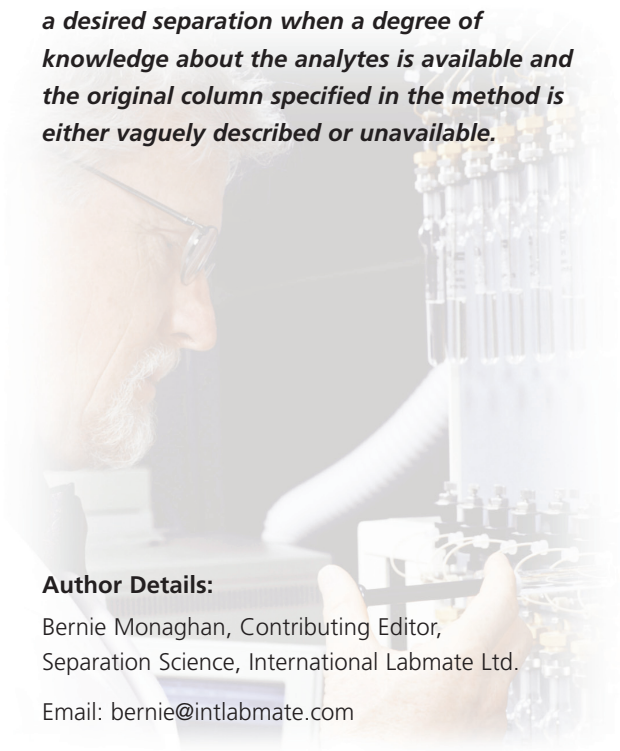
ALL C18`S ARE EQUAL – BUT SOME ARE MORE EQUAL THAN OTHERS

Bernie Monaghan

Firstly apologies to George Orwell for taking his words in vain however never has the above observation been truer than in the field of HPLC Columns containing the Octadecyl silane (C18 a.k.a ODS or RP-18) hydrophobic grouping bonded chemically to the surface of an amorphous silica surface. These are the most widely used columns in reversed phase chromatography with over 70% of chromatographers choosing this type of column for their separation, often for no better reason than its usually the one supplied with an instrument, or it is the one most likely to be lying around and available from a colleague.

Admittedly it is the most stable of all chemistries available to the chromatographer yet stability is not always the prime reason for selecting a column chemistry. So how does a chromatographer decide which C18 column to choose and, as is the norm these days, when a new C18 comes onto the market how different/close is it selectivity wise to other known C18`s? The situation is further complicated by the myriad of exotic end-capping (secondary capping) techniques employed by manufacturers these days.

More than 600 Brands on the market make it a potential minefield for the scientist if some help is not at hand to give characterisation clues to indicate the likely success of achieving a desired separation when a degree of knowledge about the analytes is available and the original column specified in the method is either vaguely described or unavailable.



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Discussed in this article are the approaches that have been utilised, and are freely available to scientists in the form of databases, compiled by independent researchers and their rationale in deciding which tests are indicative of the C18 bonding. Factors including any end-capping bonding and influence of the base silica need to taken into account when deciding the test analytes to be used to give a comprehensive profile of the various column parameters and allow meaningful comparisons between C18 columns to be made. All databases utilise specific test analytes and how they chromatograph under given conditions is indicative of certain properties of the column media.

A major problem for the chromatographer originates from one of the bibles of separation methodologies relevant to Pharmaceutical compounds and that is the US Pharmacopoeia in which the L1 Class of C18 columns for use in USP (United States Pharmacopeia) monographs are defined as "Octadecyl silane chemically bonded to porous silica or ceramic micro-particles, 3 to 10 µm in diameter, or a monolithic silica rod, " therefore assuming that all are, to a first approximation, essentially the same. Yet we have to ask are all beers or red wines the same because the carry the same tag?. Chromatographically speaking this is far from true as shown in *Figure 1*. It can be seen here that 3 columns all C18, end capped and adhering to the L1 description show different selectivity profiles and peak shapes when chromatographed under the same conditions with the same analytes. This obviously could lead to problems when substituting Column B for an assay developed and reported on Column A.

An attempt at subclassification of the group led to a list of more than 30 possibilities and was obviously going to be too confusing to users. Some better methods of comparing equivilences was clearly needed.

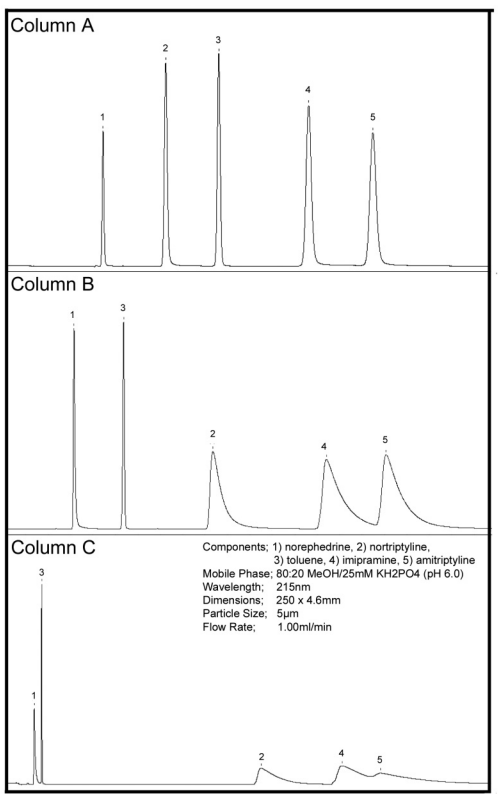

Figure 1. Comparison of 3 columns examined under identical conditions. (Reproduced courtesy of Advanced Chromatography Technologies, Aberdeen, Scotland),

This led to the different approaches as seen in various databases being developed. In fact a working group has reported[1] that a publicly available website from the USP will be ready in Autumn 2007 to help in finding possible equivilent replacements for a specific C18 column. (For details contact Margareth Marques at e-mail mrm@usp.org.)

Until then however we have the other databases, which may or may not have the column of interest to a scientist included. However several do have the experimental data available on which the scientist may perform their own characterisation experiments to determine equivalency.

Most columns contained within the databases are those produced by the commercial market leaders yet many less well-known brands are used extensively in specific geographic areas. These days of multi-national Pharmaceutical companies dictates that analytical methods are regularly transferred to laboratories in another continent where the original column may not be so freely available, hence the need for some idea of column equivalency.

USP APPROACH (UNITED STATES PHARMACOPEIA)

In 2002 the USP formed a working group consisting of members from its own Expert Committee on Pharmaceutical Analysis[2], the National Institute of Standards and Technology (NIST) and representatives from the main column manufacturing companies. The group's aim was to find a way column equivalency could be evaluated. The project started evaluating only the USP designation L1 for C18 (octadecyl silane) HPLC Columns. Subject to satisfactory completion, the work could then be extended to cover other designations e.g. L7 (Octadecyl silane), the C8 chemistries.

The group eventually decided to evaluate four column parameters using the NIST SRM 870 mix[2] which contains uracil, toluene, ethylbenzene, quinizarin and amitriptoline as a solution in methanol. The parameters measured were;

1. **Hydrophobicity:** capacity factor for ethylbenzene
2. **Chelating:** tailing factor (USP Definition) of quinizarin
3. **Activity towards bases (silanol activity):** capacity factor and tailing factor of amitriptyline
4. **Shape selectivity:** bonding density in µmols/m^2.

The rationale is that the parameters as measured by the column manufacturers are supplied to the USP who will add it to the database. The column that requires an equivalent column identifying may be deduced in order of closeness to the initial column based on one, or more, of the measured parameters according to the information logged on the database. If for example the scientist is looking for a column, which gives good chromatography for bases, then the asymmetry factor on the Amitriptiline peak is important and would be the first parameter to correlate with.

As a further aid to scientists the USP web site will have results from a second database available, the information contained in this database being supplied by PQRI and outlined in two papers published in 2004 by Snyder and co-workers[3,4]. Each set of data reported was obtained from several independent labs as opposed to the data in the USP database, which originates from the column manufacturers.

The parameters used to describe the selectivity of the various columns are;

1. **H** (hydrophobicity)
2. **S*** (steric resistance)
3. **A** (hydrogen bond acidity)
4. **B** (hydrogen bond basicity)
5. **C** (cation-exchange capacity)
6. **T** (the Type of this column describes whether the column is based on "traditional" more acidic silica gel ("A"), on "high purity", more neutral silica gel ("B"), or use a bonded phase that includes an embedded polar group ("EP").

Scientists can use either database or both to determine similarity/differences between a pair of columns. Since different parameters are used to construct the databases they cannot be combined to produce one database. However in both cases one of the columns is nominated as the reference column and a similarity factor Fs (representative of the parameters measured for that column) is set as zero.

The second columns Fs is then selected and various filters are allowed to take account of specific properties of the analytes that may be of interest. The two Fs factors are then compared. Fs factors below 3 are considered excellent matches, values below 5 are considered to be reasonable matches and above 5 poor matches.

COLUMN SELECTOR GUIDE USING PRINCIPAL COMPONENT ANALYSIS FOR COMPARISONS.

A slightly more in depth comparison database is available from ACD Labs5 (Advanced Chemistry Development Inc, Ontario, Canada) which is based on the work originally performed by Mel Euerby and Patrik Petersson[6] of Astra Zeneca and extended to include over 180 columns, most but not all of which are L1 C18 chemistries. In order to use the database it is necessary to download additional free software from the ACD Labs web site.

The database is comprised of various parameters that are measured on the columns using analytes that are chosen to reflect certain chromatographic parameters. These parameters may then be used to compare either one column versus the complete list (over 180 columns), *Figure 2* or against a specific column, *Figure 3*. Each parameter can be targeted individually so columns may be selected with high coefficients for a certain term making column switching much easier in practice.

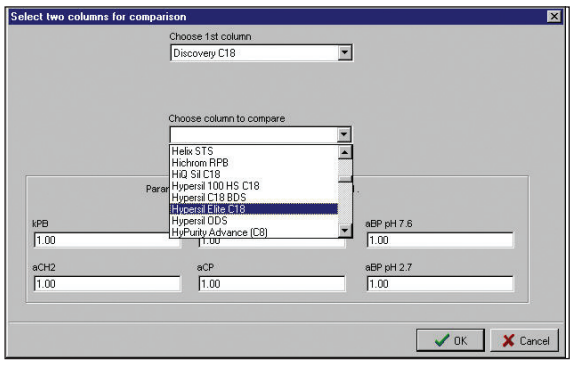

Figure 2. Screen used to compare specific column properties against rest of the database.

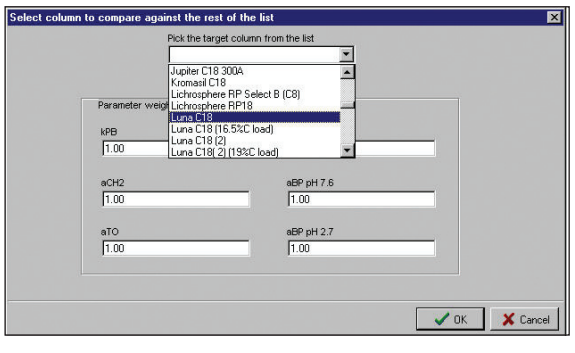

Figure 3. Screen used to compare two columns against each other.

As a further aid each parameter may be weighted for cases where certain parameters are not so important e.g. for uncharged analytes their ion exchange capacity (silanol activity) values are of no interest.

The parameters measured (and their relevance) in the construction of this database are as follows;

1. **Retention factor for pentylbenzene, kPB:** measurement of the surface area and surface coverage (ligand density)
2. **Hydrophobicity or hydrophobic selectivity:** the retention factor ratio between pentylbenzene and butyl benzene, αCH2 = kPB/kBB, is a measure of the surface coverage of the stationary phase as the selectivity between alkyl benzenes differentiated by a single methylene group depends on ligand density.
3. **Shape Selectivity, αT/O:** the retention factor ratio between triphenylene and o-terphenyl, αT/O = kT/kO, is a measure of the shape selectivity, which depends on ligand spacing and the shape/functionality of the silylating reagent.
4. **Hydrogen Bonding Capacity, αC/P:** the retention factor ratio between caffeine and phenol αC/P = kC/kP, is a measure of the number of available silanol groups and the degree of end capping.
5. **Total ion-exchange capacity, αB/P pH 7.6:** the retention factor ratio between benzylamine and phenol, αB/P pH 7.6 = kB/kP, is a measure of the total silanol activity.
6. **Acidic ion-exchange capacity, αB/P pH 2.7:** the retention factor ratio between benzylamine and phenol, αB/P pH 2.7 = kB/kP, is a measure of the acidic activity of the silanol groups.

The Chromatographic Column Selector Program contains the above parameter for 185 different columns. The mean (μ) and standard deviation (SD) for these six parameters are calculated for all columns. For each column, a normalised value (xn1 to xn6) is calculated for each of the six parameters, xnx = (xx – μ x)/SD, where xx is the value raw value for the parameter. The Euclidean distance is then used to calculate the column difference factor (CDF) between the target column and the rest of the columns. The CDF values are ranked in ascending order with the lowest CDF indicating the best column match.

SIMPLIFIED APPROACH TO CHARACTERISE RP-18`S.

Since 2001 a group of workers in Belgium and Hungary have been working on a series of characterisation tests using some of the analytes and conditions also used by other groups but in addition other tests which they use to classify and sub classify columns from different manufacturers. The database is located on the server at the University of Leuven[7].

Initial work on devising and validating data produced from a wide variety of tests in order to optimise the characterisation process was published at various symposia and in literature [8-11].

The method that was chosen was then correlated with a series of compounds of pharmaceutical interest, acetylsalicylic acid[12], vancomycin[13], and buflomedil, clindamycin, pen V, nimesulide, chloramphenicol and dihydroxystreptomycin[14]. The method was then simplified and intra- and interbatch effects were taken into account and reported[15].

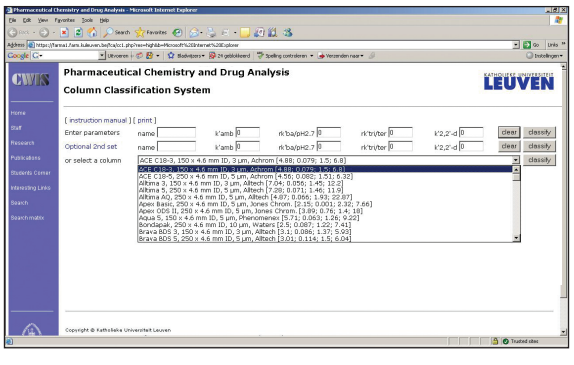

Figure 4. Screen used to compare properties of any column against one listed in database.

The method of characterisation on this database constitutes only 3 tests following the work referenced above. These methods allow the measurement of the 4 column parameters that are need for the classification. The 4 column parameters are: the retention factor of amylbenzene, k`amylbenzene, the relative retention factors of triphenylene/o-terphenyl, rk`triphenylene/o-terphenyl, the relative retention factor of benzylamine/phenol at pH 2.7, rk` benzylamine/phenol pH 2.7 and the retention factor of 2,2`-dipyridyl, k`2,2`dipyridyl .

The 4 parameter values are ascribed an F value and subsequent test columns are compared according to their diffence in F value to the reference column, with low F values indicating closest matching selectivity. Currently over 80 columns are on the database but the web site allows the parameters for two columns not on the database (see *Figure 4*) to be compared against each other. This feature is particularly useful in that it allows scientists to select columns with similar/different selectivities or columns emanating from the same manufacturer or even to view column aging during use against performance when it was new.

SUMMARY

As can be seen by looking at the various databases and academic studies reporting on column equivalency, the actual probes chosen and interpretation of the retention, peak shape and selectivity relative to other probes are important and do differ slightly from one database to another. It depends to a large degree on the knowledge of the scientist regarding the characteristics of their sample, to be able to pick out the best alternative C18 column to that cited in the USP monograph or journal article. Certainly the ever more complex innovative chemistries that are being employed by column manufacturers these days to prepare C18 Columns utilising for example embedded polar groups, secondary (and tertiary even) proprietary end capping in order to offer alternate selectivity, extra stability at high ph mobile phase or stability in highly aqueous mobile phases means that the databases are essential to scientists to allow them to make informed choices. The need for some sub classification will continue to increase.

As ever, the most asked question by novice scientists is unanswerable from the databases and that is "What's the lifetime of this column?" so we still have someway to go yet. Manufacturers always want to produce something that is similar in some respects compared to the competitors yet not something that will put them out of business by making the "everlasting C18 Column." Which database will be the first to enter the Lions Den and tabulate that information?

Acknowledgements

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