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Application of Snyder-Dolan Classification Scheme to the Selection of “Orthogonal” Columns for Fast Screening for Illicit Drugs and Impurity Profiling of Pharmaceuticals - I. Isocratic Elution

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Abstract

Fourteen judiciously selected reversed-phase columns were tested with 18 cationic drug solutes under the isocratic elution conditions advised in the Snyder-Dolan (S-D) hydrophobic subtraction method of column classification. The standard errors (S.E.) of the least squares regressions of $\log k'$ vs. $\log k'_{\text{REF}}$ were obtained for a given column against a reference column and used to compare and classify columns based on their selectivity. The results are consistent with those obtained with a study of the 16 test solutes recommended by Snyder and Dolan. To the extent that these drugs are representative these results show that the S-D classification scheme is also generally applicable to pharmaceuticals under isocratic conditions. That is, those columns judged to be similar based on the S-D 16 solutes were similar based on the 18 drugs; furthermore those columns judged to have significantly different selectivities based on the 16 S-D probes appeared to be quite different for the drugs as well. Given that the S-D method has been used to classify more than 400 different types of reversed phases the extension to cationic drugs is a significant finding.

Keywords

Column selectivity; Reversed phase; “orthogonal” separation; Snyder-Dolan classification scheme; Isocratic elution; Basic drug; Pharmaceutical analysis

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1. Introduction

Reversed phase liquid chromatography (RPLC) is one of the most powerful analytical methodologies used for drug analysis. In industry, once a routine HPLC method has been developed, it is often used for many years. As a result, the identification of a replacement or backup column with equivalent chromatographic selectivity is usually essential[1]. On the other hand, for compounds that are very difficult to separate on one column, an “orthogonal” phase with radically different selectivity is often very helpful. The use of an “orthogonal” phase can improve the resolution so that “hidden” peaks are revealed, and/or impurities are separated from the main product[1]. Also in two dimensional chromatographic separations, the column in the second dimension must be very different from the column in the first dimension to achieve high peak capacities[2]. Thus, a method for choosing similar phases for routine assays or maximally different phases for “orthogonal” separations is very important in method development.

A great deal of work has been done to try to understand column chemical selectivity [3–11]. One of the most useful and successful models is the Snyder-Dolan classification scheme based on Hydrophobic-Subtraction Model (from here on, this classification scheme will be stated as the “S-D method”) [1,12–20]. This scheme entails a quantitative description of column selectivity which allows the user to select columns with either equivalent or different selectivity; it is based on the following equation [1]:

$$\log \alpha \equiv \log \frac{k'}{k'_{EB}} = \eta' H - \sigma' S + \beta' A + \alpha' B + \kappa' C \quad (1)$$

Here, k' is the retention factor of a given solute and k'_{EB} is the retention factor of a non-polar reference solute (ethylbenzene) on the same column under the same conditions. The relative retention factor, α , is related to five terms which represent the five predominant solute-column interactions. The column properties are denoted H , S^* , A , B , and C , where H refers to column hydrophobicity, S^* refers to steric resistance, A refers to hydrogen-bond acidity, B refers to hydrogen-bond basicity, and C refers to cation-exchange activity. The corresponding complementary solute properties are denoted as η' , σ' , β' , α' , and κ' . The values of the selectivity parameters H , S , etc. obtained for a given column under one set of conditions (%B, temperature, solvent type, etc.) can be used to characterize that column's selectivity for other conditions. The five column-selectivity parameters (H , S^* , etc.) are of primary practical interest because they have been used to determine the selectivity and applicability of more than 400 RPLC columns [1,12,21]. Application of this model can give a better understanding of the nature and relative importance of different solute-column interactions. For example, the practical importance of these five contributions to column selectivity for type-B alkylsilica columns increases in the order: $H < B < S^* < A \ll C$ [15]. However, Marchand et al. observed that B and particularly C are major contributors to values of F_s when F_s is greater than about 10 [22]. Therefore, for basic (cationic) solutes, the silanol acidity (A) and charge-charge (C) interactions are of major importance in determining column selectivity, while H makes a lesser contribution to column selectivity.

The advantage of this model is that a single parameter called the “column selectivity function, F_s ” has been defined as in Eq. (2) [1]; F_s can be used to quantitatively compare the overall selectivity of any two columns.

$$F_s = \{ [12.5(H_2 - H_1)]^2 + [100(S_2 - S_1)]^2 + [30(A_2 - A_1)]^2 + [143(B_2 - B_1)]^2 + [83(C_2 - C_1)]^2 \}^{1/2}$$

Each term of Eq. (2) is weighted differently because the column parameters (H , S^* , etc.) vary in their relative contribution to the overall selectivity [1,15]. The F_s value can be considered as the distance between the positions that two columns occupy in the 5 dimensioned coordinate system defined by the 5 phase parameters, modified by multiplicative factors (12.5, 100, etc.) which help to weight each of the 5 contributions to the overall column selectivity more equally. Clearly, the smaller is the weighted distance (i.e., F_s), the more similar are the two columns. In the extreme case when two columns are sufficiently close ($F_s \approx 3$), two columns can be considered essentially “equivalent” [1,15]. On the other hand, columns with bigger F_s values are more widely separated in 5-D space; correspondingly they are more different in terms of H , S^* , etc., and therefore in terms of selectivity.

Based on the use of 16 judiciously selected but chemically simple probe solutes Snyder, Dolan, et al. have characterized the selectivity of over 400 commercial reversed phase columns using the 5 parameters by fitting the measured retention data of these 16 solutes to the Eq. (1)[1,12–20]. The resulting F_s values can be used for easy selection of phases that should be nearly interchangeable, and conversely, phases that are dramatically different. These are the most important practical applications of the S-D method.

However, in developing methods for pharmaceutical analysis, impurity profiling, and in forensic analysis of intoxicants, the sample solutes are usually drugs with very different structures and properties. For example, most drugs of abuse are rather hydrophilic basic drugs of quite different structures. These highly variegated compounds might for various reasons exhibit interactions not encompassed by those in S-D 16 rather simple solutes which include only two basic drugs each containing only a single basic site and they were used to study the wide variety of phases that constitute the 400 odd phases studied by S-D. The early work of the S-D group used 90 diverse solutes [12,14]; however, only 10 type-B alkylsilica phases were studied. Therefore, it is still, in our opinion, an open question as to whether the S-D method will work for a wide variety of drug solutes with columns other than type-B alkylsilica. The chief purpose of this work is to assess whether the S-D method can predict the equivalence and non-equivalence of columns for basic drugs.

There is a strong need for “orthogonal” separation conditions in the pharmaceutical industry to ensure that the final method will detect process impurities and degradants in the pharmaceutical material. Many have found these “orthogonal” methods extremely useful when incorporated into a method development screening process [23–30]. To achieve “orthogonal” separation conditions in RPLC, the stationary phase [28–34] and mobile phase (in terms of the pH [28–30,34], type of organic modifier [29,34] or lipophilic additive [35]) will need to be changed. Variations in the stationary phase type [34,36–38], in the mobile phase pH [28–30,34,36,39], and type of modifier [34,39] generally have the largest effects on selectivity while changes in the temperature, or gradient steepness are usually done last for more fine tuning of retention and selectivity. As an alternative to changing the stationary phase and mobile phase, others have used separation techniques other than RPLC such as capillary electrophoresis (CE) [40], supercritical fluid chromatography (SFC) [23] and hydrophilic interaction chromatography (HILIC) [38] to find an “orthogonal” separation.

There has been a great deal of effort devoted to developing column classification schemes and applying them to choose the best conditions for a pharmaceutical analysis. For example, several groups have used the S-D method to choose an appropriate column for a given pharmaceutical separation problem or to replace columns used in routine pharmaceutical separations [24,32]. Hoogmartens and coworkers have developed a column ranking system based on a four parameter model derived using principal component analysis (PCA) and have found this model useful in choosing the appropriate column for numerous drug

methods [24–27,30,41–45]. They also used this model to investigate changes in the stationary phase selectivity resulting from routine use or long-term storage of the column [46]. Euerby [47] developed a six parameter column classification scheme using PCA based on Tanaka's method [48]. Heyden et al. used chemometric techniques (such as PCA, OPA and others) and found that their chemometric methods were able to choose a more refined list of columns for use in drug analysis as those most likely to be chosen based on general chromatographic knowledge alone [33,49]. Others have used quantitative structure-retention relationships (QSRR) to obtain a better understanding of what column and stationary phase parameters most strongly influence the selectivity and retention for basic drugs [41,42]. Despite all of this effort for characterizing column selectivity and locating "orthogonal" methods, it appears that no one has explicitly tested the ability of the S-D model to predict the equivalence and non-equivalence of columns for basic drugs with a wide variety of column types.

In this work, we tested the S-D method to see if it can successfully predict similarities and differences in stationary phase behavior for a wide variety of prototypical drug substances under isocratic conditions, and more importantly, to apply the S-D method to find "extreme" phases for "orthogonal" separations, fast drug screening, and impurity profiling. A set of fourteen columns in four groups (shown in Table 1) was selected based on their S-D F_s values. The first three groups which are different from one another are each comprised of reversed phases that are similar in terms of their S-D parameters; while the phases in the fourth group are extremely different from one another and from the other classes. All columns were first tested with the 16 S-D test solutes to make sure that these columns are well fit by the S-D solute parameters and that the columns were behaving properly. These columns were then tested with 18 drug substances including benzodiazepines, antihistamines, and antidepressants under S-D isocratic pH 2.8 conditions.

We used two approaches to determine whether the S-D database would allow us to select similar or distinct columns for analysis of drug substances. The first approach used the standard error (S.E.) of a linear fit of the $\log k'$ on a test column vs. $\log k'$ on a reference column, as was done by S-D in their original development of Eq. (2)[12]. An alternative approach, based on the recognition that the S-D model predicts that selectivity can be represented by a 5-dimensional model (as shown in Eq. (2)), is to simply use a 5 component principal components analysis (PCA) model to represent the experimental $\log \alpha$ data, for both the S-D and drug solutes. This approach allows the similarities and differences of columns to be visualized, both in the form of score plots and in the form of dendrograms. .

2. Experimental

2.1. Reagents

HPLC grade acetonitrile (ACN) and isopropyl alcohol (IPA) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade water was prepared in-house from a Barnstead Nanopure II deionizing system (Dubuque, IA). This water was boiled to remove carbon dioxide and passed through a 0.45 μm nylon filtration apparatus (Lida Manufacturing Inc., Kenosha, WI) immediately before use. Phosphoric acid (89 % w/w) and monobasic potassium phosphate were obtained from Aldrich. All eluents were prepared gravimetrically (± 0.01 g) based on the density (at 25 °C) of acetonitrile (0.778 g/mL) and water (0.998 g/mL) where eluent composition is reported as the v/v ratio. The stock 60 mM potassium phosphate pH 2.8 buffer solution was prepared by mixing 60 mM phosphoric acid in water and 60 mM monobasic potassium phosphate in water at a ratio of 100/435.38 (w/w). The mobile phase was 50/50 (v/v) acetonitrile/60 mM pH 2.8 buffer for all experiments. The phosphate concentration in final mobile phase was 30 mM.

2.2. S-D solutes and drug standards

S-D solutes (uracil, acetophenone, benzonitrile, anisole, toluene, ethylbenzene, 4-nitrophenol, 5-phenylpentanol, 5, 5-diphenylhydantoin, chalcone, N, N-dimethylacetamide, N, N-diethylacetamide, 4-n-butylbenzoic acid, mefenamic acid, nortriptyline, and amitriptyline) were of reagent grade or better and were used as obtained from Sigma-Aldrich without further purification. Standards of the amphetamine, opiate, and benzodiazapine drugs studied in this work were obtained from the Minnesota Bureau of Criminal Apprehension (BCA) as 1 mg/mL stock solutions in methanol (originally from Cerilliant (Round Rock, TX, USA)). All antihistamine and antidepressant drugs were purchased from Sigma-Aldrich as solids and were used without further purification. 18 drugs were chosen as probe solutes from a list of over 70 drugs evaluated based on their retention times and UV spectra.

Stock solutions of each S-D solute, antihistamine or antidepressant drug purchased as a solid were made at 1 mg/mL using methanol or acetonitrile as the diluent. Analytical samples of S-D solutes or the drugs were prepared by diluting the stock solutions to 50 µg/mL with the isocratic mobile phase (50/50 (v/v) acetonitrile/buffer). Five drug mixtures were prepared by appropriate dilution of the stock drug solutions with the isocratic mobile phase to yield a final concentration of each drug at 50 µg/mL.

2.3. Columns

The columns used in this work are listed in Table 1. Columns were 50 mm × 4.6 mm I.D. with 5 µm particles. Except for the Inertsil CN and the Hyper-Crosslinked columns, all other columns were generous gifts of the companies listed in Table 1. The hydroxylated Hyper-Crosslinked phase (denoted HC-OH) and sulfonated Hyper-Crosslinked C8 phase (denoted HC-SO₃⁻) were synthesized and packed in-house by methods described in the literature [50,51]. An Inertsil CN column (150 × 4.6 mm I.D.) was purchased from GL Science and the particles were unpacked from this column and repacked into a 50 mm × 4.6 mm I.D. column using the packing procedure from our previous papers [50,51]. The stainless steel column hardware was obtained from Isolation Technologies (Hopedale, MA).

2.4. Instrumentation and chromatographic conditions

All chromatographic experiments were carried out on an HP1090 chromatography system controlled by version A.10.01 ChemStation software (Agilent Technologies; Palo Alto, CA). This instrument was equipped with an autosampler, a temperature controller, and a photodiode array UV detector (DAD). The “intrinsic” dwell volume of the instrument, including all tubing required to connect the column, was measured as 1.084 mL using the method found in chapter 4 of Ref [52].

Prior to measurements, each column was flow-equilibrated for 60 min with mobile phase (Several columns (Prism, Bonus and Inertsil CN) were examined after equilibrating for 60 minutes and again after more than 18 hours. No changes in retention were observed for ethylbenzene, halazepam, prazepam, butyl benzoic acid or mefenamic acid. We believe at all the S-D parameters reported here, except possibly for the ZirChrom PS phase, are quite stable with time). The conditions for determining the isocratic retention time of the solutes on each column were: a flow rate of 1.0 mL/min, an injection volume of 5 µL (unless otherwise stated), a detection wavelength of 210 nm (for dimethylacetamide and diethylacetamide) or 254 nm, and a column temperature of 35 °C. The UV spectrum of each analyte from 200–400 nm was collected to confirm identification of each drug when running the drug mixtures.

2.5. Data analysis

Data were collected and processed using ChemStation software. Retention times obtained by ChemStation were exported to Microsoft Excel (using a macro from the Agilent User Library of Macros) for further analysis. For isocratic measurements, the retention factors (k') were determined for each solute and each column using Eq. (3):

$$k' = \frac{(t_R - t_0)}{t_0} \quad (3)$$

where t_R and t_0 are the retention time of a given solute and of uracil both corrected for the extra-column dead time of the system. In general each sample mixture was injected 3 times and the average result was used in subsequent calculations. As done for the S-D studies, the standard error (S.E.) in plots of $\log k'$ for condition 1 versus $\log k'$ for condition 2 was used to gauge the selectivity difference. The precision of the measurement of k' is such that for replicate injections of the same solutes on the same column random experimental errors give S.E. values of 0.002 to 0.003. The smaller the value of S.E., the more qualitatively similar the two columns in the group are in terms of selectivity, and vice versa.

Principal components analysis (PCA) was performed and dendograms were prepared using the Matlab programming environment, v. R2007b (Mathworks Inc., Natick MA) using the PLS Toolbox, v. 4.1 (Eigenvector Research, Inc., Wenatchee, WA). The $\log k'$ data for the S-D and drug solutes were mean-centered in both the row and column directions, and analyzed using PCA, assuming 5 components, to be consistent with the 5-dimensional model used in Eq. (2).

3. Results and Discussion

3.1 Column selection

We selected 12 reverse phase columns based on careful inspection of the 400 phases in the S-D collection and their relative F_s values [53]. In addition, two phases (HC-OH and HC-SO₃⁻) developed in our laboratory were also chosen for investigation. As shown in Table 1, the first three groups (see groups I, II, and III) contain phases of similar functionality within each group but the selectivities differ between groups. Phases in group IV are very different from one another and from all the other columns (i.e., “orthogonal”). The similarity and difference of these columns were also verified with our stationary phase triangle scheme [54] to ensure that we chose representative groups of columns. These columns were used to study column selectivity for a wide variety of drug solutes under isocratic elution.

3.2 Validating the test columns with the S-D 16 solutes

All columns were tested with the standard 16 solutes under the standard isocratic conditions to see if they were well fit by the S-D method, using the solute parameters, to assure that the columns were behaving properly and to check to see that the phase parameters agreed with the recommended values [1]. The parameters (H , S^* , A , B , C) reported in Table 2 were obtained by a least-squares fit of our measured $\log k'$ values for the 16 solutes to Eq. (1) using the S-D solute parameters (η' , σ' , β' , α' , κ') [1]. The differences between our measured phase parameters and the S-D values are given in Table 3. We can see that, overall, the measured parameters for columns in groups I and II are rather close to the parameters given by S-D, while the measured parameters for the ZirChrom PS, the Nova Pak CN, and the Bonus RP columns in groups III and IV are different from the S-D parameters. In addition, the values for the A and C terms differed significantly for many of the columns. These differences in the phase parameters are possibly due to the fact that our columns are not the same as those used by Snyder and in some cases the columns were used

over a considerable period of time testing them with drugs before the phase parameters were determined. However, Marchand et al have noted that the A and C parameters for several phases vary upon storage in acetonitrile/water to greater extent than the other three parameters [22].

Fig. 1A shows a plot of $\log k'$ (measured) vs. $\log k'$ (calculated) for a Zorbax SB C18 column. Here, $\log k'$ (measured) is our measured values for the 16 S-D solutes on the Zorbax SB C18 column while $\log k'$ (calculated) were calculated from Eq. (1) based on S-D solute parameters and column parameters. The S.E. of this plot is the standard error of the least squares fit of $\log k'$ (measured) and $\log k'$ (calculated). Fig. 1B gives the plot of S.E. as obtained in Fig. 1A for 11 columns vs. the S.E. reported by S-D for the fit of their $\log k'$ to Eq. (1). The correlation is not very strong ($r^2 = 0.877$, S.E. = 0.03) even when the Nova Pak CN column was excluded for the regression, which tell us that the measured $\log k'$ values on all other columns do not follow the model very well if the S-D phase parameters and solutes are used (It is unclear why the Nova Pak CN phase is so far off the line compared to all the other columns). However, when we fit our measured values of $\log k'$ to Eq. (1) using only the standard solute parameters and allowed the least squares program to adjust the phase parameters, the resulting S.E. values, with the exception that for the Nova Pak CN phase become very close to the S.E. values reported by S-D for the same phase in their original work (Table 3). There is a much improved correlation ($r^2 = 0.974$, S.E. = 0.009, see Fig. 2). In other words, our measured $\log k'$ values are fit very well by the S-D model when our measured column parameters and S-D solute parameters are used. We believe that this strongly suggests that the deviations shown in Fig. 1B are *not* due to the failure of the model but are most likely due to batch-to-batch variation in phases or to column ageing.

3.3 Analysis of column similarity based on the S-D standard solutes

We checked our S.E. values against the F_s values using the Discovery C18 as a reference column. We first compute the set of F_s values for all phases relative to the reference column. Next, for each column we determine the S.E. for a least square regression of $\log k'$ on the test column vs. $\log k'$ on the reference column. A plot of S.E. vs. F_s is shown in Fig. 3A. As seen the correlation is weak ($r^2 = 0.474$, S.E. 0.15). This is primarily due to the ZirChrom-PS phase (circled). We note that the F_s values were computed from Eq. (2) using the column parameters reported by S-D (except for the HC-OH and HC-SO₃⁻ phases). When we compute F_s based on the column parameters determined in this work (see Fig. 3B) there is a vast improvement in the quality of the correlation ($r^2 = 0.961$, S.E. = 0.04).

3.4 Analysis of column similarity based on drugs

The $\log k'$ values for the selected 18 drug solutes were used to compare the selectivity of all columns under isocratic conditions. Fig. 4A and 4B give $\log k'$ plots for Alltima C18 and Prism RP columns against our reference column (Discovery C18). The very strong correlation ($r^2 = 0.9994$, S.E. = 0.0088) between Alltima C18 and Discovery C18 indicates that these two phases are extremely similar, while the very weak correlation ($r^2 = 0.6848$; S.E. = 0.267) observed between Prism RP and Discovery C18 showed that these two phases have very different selectivities for these drugs. These relationships are also illustrated in Fig. 5 which shows chromatograms for the separation of several selected drugs. A good separation of these solutes is obtained on Discovery C18 and Alltima C18. Also, Alltima C18 provides a very similar elution profile for these solutes compared to Discovery C18 which is consistent with the small F_s value (9.7) between Alltima C18 and Discovery C18. However, the retention and elution order of these solutes on Prism RP are very different from Discovery C18, again consistent with the very large F_s value (252.3) between Prism RP and Discovery C18.

The S.E. values for the pairings of the 14 columns are summarized in Table 4. It should be noted that the values above the diagonal will not be the same as the values below the diagonal. This is due to the fact that for dissimilar columns the S.E. of the regression for column A vs. column B will not be the same as the S. E. for the reverse operation because the slope of the plot is not unity.

Based on our experience, we conclude that two columns are 1) very similar if $S.E. < 0.05$ ($F_s < 16$); 2) similar if $0.05 < S.E. < 0.10$ ($16 < F_s < 35$); 3) reasonably different if $0.10 < S.E. < 0.15$ ($35 < F_s < 55$) and 4) rather different if $S.E. > 0.15$ ($F_s > 55$). The four C18 phases in group I of Table 1 are all very similar ($S.E. = 0.01 - 0.05$) to one another. The three phenyl phases in group II of Table 1 are similar ($S.E. = 0.06 - 0.09$) and also similar ($S.E. = 0.04 - 0.11$) to the C18 phases of group I. The Prism RP and Bonus RP in group III of Table 1 are similar ($S.E. = 0.06$) but very different from all other phases ($S.E. = 0.16 - 0.48$), with the exception that the Prism RP is similar to Inertsil CN ($S.E. = 0.08$). All columns in group IV (Nova Pak CN, Inertsil CN, ZirChrom PS, HC-OH, and HC-SO₃⁻) are very different from one another ($S.E. = 0.15 - 0.52$) and also very different from the phases in group I, II. ($S.E. = 0.15 - 0.52$). In addition, we can see that the Nova Pak in group IV is about the same distance from all other phases, which makes it one of the most unusual of the 14 phases examined. The HC-OH phase is also quite distinctive because it is nearly equidistant from all columns to which it is compared. The sulfonated phase is extremely different from all other columns ($S.E. = 0.42 - 0.52$), making it by far the most interesting phase. It will likely be very useful as an “orthogonal” column most especially for basic drugs.

Fig. 6 shows a relationship between the S.E. values of the log k' plots for the 18 drugs (Discover C18 was used as the reference column) and the F_s values, based on our determination of the phase parameters (not the values of Snyder), for the 16 S-D solutes. The regression parameters for this relationship are:

$$S.E._{drugs} = 0.0026 (\pm 0.0002) F_{s, \text{present study}} + 0.008 (\pm 0.016); r^2 = 0.943; S.E. = 0.04 \quad (4)$$

The correlation ($r^2 = 0.943$; $S.E. = 0.04$) indicates that column groupings based on the 18 drugs are consistent with our measured F_s values. This correlation of S.E. and F_s obtained for 18 drugs and various types of columns is much better than the one derived by S-D [15] for 16 S-D solutes and type-B alkylsilica columns ($S.E._{S-D \text{ solutes}} = 0.0036 F_{s, S-D} + 0.002$; $r^2 = 0.72$; $S.E. = 0.002$).

Fig. 7 shows a plot of the S.E. values for the 18 drugs vs. the S.E. values for the 16 S-D solutes obtained for all columns with Discovery C18 as the common reference phase. The reasonably strong correlation ($r^2 = 0.898$, $S.E. = 0.06$) indicates that the column selectivity for the drugs is well represented by the column selectivity defined using the S-D 16 solutes. It is actually rather striking that these two correlations are so strong given the very different natures of the solutes in each of these sets. Taken together they suggest that the original 16 solutes of the S-D method do a very good job in general of representing the behavior of drugs despite the fact that the S-D data set contains only two bases.

All of the above results indicate that the column groupings for the drugs are very similar to the original groupings based on the F_s values generated using solutes not necessarily representative of pharmaceutical samples. Therefore, the S-D method based on its standard 16 solutes does a reasonably good job of predicting the classification of these columns if we were to use drug solutes. That is, columns predicted to be similar are similar and columns predicted to be very different are indeed radically different, when drugs are used to characterize the columns.

3.5 Analysis of column similarity based on drugs – analysis using euclidean distances

As an alternative to using the S.E. analysis described above, to evaluate how well the analysis of the S-D solutes can be used to predict column similarities and differences for drug solutes, we calculated the distances in 5 dimensional space using the $\log k'$ values. First, principal component analysis (PCA) was used to create a 5 component (i.e., 5 dimensional) model for the $\log k'$ values. The virtue of the PCA method is two fold. First, it is mathematically guaranteed to produce the lowest residual variance for a given number of components; second, each successive factor is maximally “orthogonal” to its predecessor. Next, score plots and dendrograms to visualize the relationships between the columns, for both the S-D and drug solutes. The score plots are based on only the first two most important factors but the dendrograms are based on the first five factors. All plots are shown in Fig. 8. Table 5 shows the S.E. values for various PCA models from one to five components for both the S-D solutes and the drug solutes. The pooled S.E. value for the fits of the S-D solutes to Eq. (1) was 0.075, demonstrating that the five component PCA model is better than the 5 factor S-D model of Eq. (1). This is expected as PCA by its chemically unconstrained nature is designed to produce the smallest possible variance $((S.E.)^2)$. It is most interesting to note that the PC models for the S-D solutes are in each case significantly better ($< 5\%$ possibility of being a random result) than the PC models for the drug solutes for each model, except for the one component model, where there is no significant difference. This suggests that the drug solutes span a larger range in solute behavior than do the S-D solutes. This may be a consequence of π - π and dipole-dipole interactions which show up in phenyl [18] and cyano [19] phases. It could also be due to the fact that some non-type-B alkylsilica phases (e.g. zirconia based phase) are just not as well fit as type-B alkylsilica [16].

We first consider Fig. 8A which shows the score plot for the S-D solutes. As can be seen, the columns in Groups I, II and III cluster in a fashion very much as one expects based on the S-D scheme, while the Group IV columns are widely distributed across the PC space again as expected based on the S-D scheme. This behavior is reasonably consistent with our analyses discussed above based on the S-D F_s parameter and S.E. plots. Alternatively, we can examine the columns by examining the dendrograms, as shown in Fig. 8B. The distance between columns is shown on the x-axis; the columns that are most similar are joined together in the plot at the corresponding distance. In this case, the Alltima C18 and ACE C18 columns are the most similar, with a distance of 0.09; hence, the lines corresponding to these two columns are joined at a distance of 0.09. The phenyl phases (Group II) group together at a distance of 0.20, and all the Group I phases group together at this distance as well. This procedure continues until the last two groups/columns are connected. Upon inspection of the plot, we see that at a distance of about 0.30 (represented by the dashed vertical line), the columns in Groups I, II and III are now organized into their individual groups, and all of the other, dissimilar, columns each form their own group. This observation fully supports our choice of these column groupings, and is agreement with our conclusions using the S-D hydrophobic subtraction model.

Fig. 8C and 8D show the analogous score plots and dendrogram plots based on the 18 drug solutes. Again, in the score plot (Fig. 8C), we see that the columns cluster appropriately into their respective groups (for Groups I, II and III), and the Group IV columns are distributed across the PC space. The locations of the groups and columns are *somewhat different* from those observed for the S-D solutes (Fig. 8A). Some of these differences can be attributed to the fact that we are only visualizing the relationships in two dimensions instead of five dimensions. It also can be seen that for the drug solutes, the Group I and Group II columns are not as well separated by the drugs as by the standard S-D solutes.

The dendrogram plot for the drug solutes is shown in Figure 8D. While the same *general* clustering behavior is seen for the drug solutes as was observed for the S-D solutes, the clusters based on the drug solutes are not as well differentiated as by the S-D solutes. It seems that S-D are quite perspicacious in their choice of the 16 standard solutes. Of note for both the S-D and drug solutes, the *most unique* column behavior is seen for the ZirChrom PS and HC-SO₃⁻ phases. Therefore, the *general* if not the detailed trends in similarities and differences are maintained by the 18 drug solutes as compared to the 16 S-D solutes.

Another means of determining how well the S-D solute selectivities are able to track the drug solute selectivity is shown in Fig. 9. The pair wise distances for all column pairs are plotted in this figure, with the drug solute-based distances on the y-axis and the S-D solute-based differences plotted on the x-axis. The regression parameters for this relationship are:

$$D_{\text{drugs}} = 0.78 (\pm 0.05) D_{\text{S-D}} + 0.16 (\pm 0.09); r^2 = 0.737; \text{S.E.} = 0.44 \quad (5)$$

The correlation coefficient for this regression is 0.737, which indicates only modest agreement between the similarities and differences predicted by the two solute sets. The standard error of 0.44 indicates that the S-D solutes do not predict the details of small differences between the columns when analyzing drug solutes. The most significant outlier on this plot (circled on Fig. 9) corresponds to the ZirChrom PS/HC-SO₃⁻ column pair. In fact, three other obvious outliers also corresponded to column pairs involving the ZirChrom PS phase. We therefore eliminated the ZirChrom phase from the analysis and found the following regression parameters:

$$D_{\text{drugs}} = 0.94 (\pm 0.04) D_{\text{S-D}} - 0.01 (\pm 0.06); \text{S.E.} = 0.30; r^2 = 0.877 \quad (5)$$

The difference in the S.E. upon omitting the ZirChrom phase from the analysis is significant at $p = 0.047$. In addition, upon omitting the ZirChrom phase from the analysis, the slope of the regression equation becomes statistically unity 1 and the intercept not significantly different from zero. This result suggests that there may be some unique interactions of the drugs with the ZirChrom PS column that are not probed by the S-D solutes. Alternatively, the well known [55] dependence of zirconia phases on their extent of exposure to phosphate containing eluents may also be the cause of a transient change in their behavior [56]. However, S-D noted a higher than typical S.E. of fits on zirconia-based and polymer coated phases [16].

In preliminary work, we used Eq. (1) to estimate solute parameters for the drug solutes. For both η' and κ' parameters we found that the range of values spanned by the solute parameters by the drug solute set was similar to the range observed for the S-D solutes. The steric parameters (σ') for the drug solutes span a larger range than do the S-D solutes. This effect may be responsible for the increased separation of the HC-OH phase (a phase with a large S^* parameter) from the Group II phases for the drug solutes, as seen by comparison of Figures 8A and 8B (S-D solutes) and Figures 8C and 8D (drug solutes). In the case of the β' parameter, if we excluded the acetamides from consideration, we find that the drug solutes span a larger range of β' values as compared to the S-D solute set as well, however, there does not seem to be any clear evidence for this effect in the plots of Figure 8. The α' values are not at all well-predicted using any combination of columns we have tried thus far, so these suggestions are preliminary.

4. Conclusions

In this study, we have tested and compared the selectivity of 14 columns for 18 drugs and the set of 16 S-D solutes under the S-D conditions and conclude that:

1. The results from both S.E. method and independent 5 component PCA method showed that the S-D method based on its standard set of 16 test solutes does allow one to choose columns with equivalent and distinct selectivities for the drug solutes. That is, those columns judged to be similar based on the S-D 16 solutes were similar based on the 18 drugs; furthermore the different columns are indeed rather different in term of the drug solutes; however, it is not suitable for predicting minor differences in selectivity (S.E. values < 0.008 to 0.012) between very similar columns.
2. The sulfonated phase is the most extreme phase compared to all other phases in the four groups. It ought to find significant application as an “orthogonal” phase in pharmaceutical analysis.
3. The general features of the column similarities and differences are preserved when using Euclidean distances based on the drug solutes to compare the columns; however, the clustering of similar phases is not as tight as is observed when using the S-D solutes.
4. There may be a unique interaction not reflected in the S-D solutes or the S-D model needed to describe the retention of the drugs on the ZirChrom PS column.

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Appendix A

Retention times of 18 drugs on 14 columns under isocratic conditions

Drugs	Discovery C18	SB C18	ACE C18	Alltima C18	Zorbax Phenyl	BetaBasic Phenyl	Prontosil Phenyl	Prism RP	Bonus RP	Inertsil CN	Nova Pak CN	ZirChrom PS	HC-OH	HC-SO3
Aminoflunitrazepam	0.740	0.636	0.735	0.734	0.855	0.870	0.882	0.844	0.650	0.764	0.652	1.868	1.373	1.114
Desipramine	0.824	0.674	0.826	0.816	0.761	0.942	0.822	0.614	0.493	0.649	0.805	1.899	1.042	18.949
Perphenazine	0.852	0.717	0.851	0.846	0.733	0.947	0.803	0.614	0.492	0.646	0.851	1.823	1.382	14.273
Amitriptyline	0.905	0.751	0.924	0.894	0.853	1.017	0.931	0.564	0.462	0.644	0.705	2.536	1.167	25.186
Bromazepam	0.845	0.764	0.875	0.845	0.865	0.911	0.913	1.022	0.763	0.790	0.697	2.006	1.112	1.848
Oxazepam	0.972	0.877	1.042	0.964	0.921	0.986	1.029	1.339	0.817	0.957	0.736	1.868	1.117	1.114
Nitrazepam	1.030	0.949	1.119	1.029	1.092	1.017	1.211	1.352	1.032	0.815	0.925	4.867	0.776	1.274
Estazolam	0.972	0.993	1.042	0.964	1.298	1.050	1.213	1.139	0.938	0.842	0.652	1.868	1.373	1.820
Triazolam	1.077	1.192	1.183	1.038	1.451	1.136	1.314	1.268	0.881	0.867	0.816	2.283	0.802	6.508
Nordiazepam	1.239	1.233	1.405	1.242	1.181	1.145	1.326	1.612	1.209	0.945	0.697	9.093	1.311	1.424
Flunitrazepam	1.248	1.249	1.420	1.244	1.728	1.279	1.670	1.499	1.184	0.972	0.720	6.812	1.382	1.493
Temazepam	1.268	1.255	1.444	1.268	1.235	1.210	1.444	1.556	1.143	0.917	0.693	2.063	1.928	1.445
Clobazam	1.359	1.354	1.567	1.361	1.403	1.307	1.620	1.513	1.184	0.961	0.705	3.830	1.329	1.434
Lormetazepam	1.361	1.367	1.565	1.358	1.437	1.271	1.511	1.691	1.235	0.957	0.807	2.485	1.373	1.519

Drugs	Discovery C18	SB C18	ACE C18	Alltima C18	Zorbax Phenyl	BetaBasic Phenyl	Prontosil Phenyl	Prism RP	Bonus RP	Inertsil CN	Nova Pak CN	ZirChrom PS	HC-OH	HC-SO3
Diazepam	1.701	1.912	2.040	1.697	1.710	1.416	1.981	1.924	1.531	0.996	0.747	2.063	1.321	2.451
Halazepam	3.060	3.485	3.925	3.088	2.529	2.022	3.185	3.140	2.739	1.244	0.857	5.316	1.728	2.451
Prazepam	3.026	3.703	3.852	3.023	2.670	1.989	3.299	3.170	2.716	1.197	0.816	11.073	2.312	3.633
Bucizine	4.059	4.578	5.025	3.970	3.378	2.636	4.394	4.174	3.381	1.385	0.823	8.703	3.644	4.007
Uracil	0.562	0.433	0.507	0.561	0.471	0.624	0.472	0.488	0.438	0.570	0.546	1.606	0.623	0.490

Appendix B

The structures of 18 drug substances

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Fig. 1.

(A) Plot of $\log k'$ (measured) vs. $\log k'$ (calculated) for SB C18 column. $\log k'$ (calculated) were obtained from Eq. (1) based on S-D solute parameters and column parameters. The S.E. of this plot is the standard error of the least squares fit for 16 S-D solutes. It is denoted S.E.(this work). ($\log k'_{\text{measured}} = 0.972(\pm 0.008) \log k'_{\text{calculated}} + 0.006(\pm 0.004)$; $r^2 = 0.999$; S.E. = 0.017); (B) Plot of S.E. as obtained in Fig. 1A for 11 columns vs. the S.E. reported by S-D for the fit of their $\log k'$ to Eq. (1). ($\text{S.E.}_{\text{this work}} = 1.3 (\pm 0.2) \text{ S.E.}_{\text{S-D reported}} + 0.03 (\pm 0.01)$, $r^2 = 0.877$, S.E. = 0.03). Circled point is for Nova Pak CN, which is excluded for the regression. Chromatographic conditions: 50% ACN/buffer (30 mM, pH 2.8); 35 °C; 1.0 mL/min

Fig. 2.

Plot of S.E. for the fit of measured log k' with measured H, S, A, B, C values in equation (1) vs. S.E. reported by S-D. (S.E.-fitted with measured H, S*, etc. = $0.98 (\pm 0.06)$ S.E. S-D reported + $0.005 (\pm 0.004)$, $r^2 = 0.974$, S.E. = 0.009). Circled point is for Nova Pak CN, which is excluded for the regression. Other conditions as stated in Fig. 1.

Fig. 3.

(A) Plot of S.E. (S-D solutes) vs. F_s (S-D) for 12 columns. The S.E. (S-D solutes) is the standard error of the least squares regression of $\log k'$ for S-D solutes on the test column vs. $\log k'$ on the reference column (Discovery C18). F_s (S-D) were calculated from Eq. (2) based on S-D column parameters H , S^* , etc. of the given column against the Discovery C18. ($S.E._{S-D \text{ solutes}} = 0.0013 (\pm 0.0004) F_{s, S-D} + 0.07 (\pm 0.06)$; $r^2 = 0.474$; S.E. = 0.15). (B). Plot of S.E. (S-D solutes) vs. F_s (present study) for all 14 columns. F_s (present study) were calculated based on our measured H , S^* , etc. of the given column against the Discovery C18. ($S.E._{S-D \text{ solutes}} = 0.0034 (\pm 0.0002) F_{s, \text{present study}} - 0.01 (\pm 0.02)$; $r^2 = 0.961$; S.E. = 0.04). Circled points are for ZirChrom PS. Other conditions as stated in Fig. 1.

Fig. 4. Comparisons of column selectivity using 18 drug solutes. (A) log k' plot of Alltima C18 vs. Discovery C18. ($\log k'_{\text{Alltima}} = 1.007 (\pm 0.006) \log k'_{\text{Discovery}} - 0.005 (\pm 0.002)$; $r^2 = 0.9994$; S.E. = 0.0088). (B) log k' plot of Prism RP vs. Discovery C18. ($\log k'_{\text{Prism}} = 1.06 (\pm 0.18) \log k'_{\text{Discovery}} + 0.127 (\pm 0.064)$; $r^2 = 0.6848$; S.E. = 0.267). Other conditions as stated in Fig. 1.

Fig. 5. Chromatograms of selected drugs on three columns (Discovery C18, Alltima C18, and Prism RP). Samples: (1). 7-aminoflunitrizepam; (2). perphenazine; (3). clonazepam; (4). temazepam; (5). lorazepam; (6). diazepam; (7). halazepam; (8). Buclizine. Other conditions as stated in Fig. 1.

Fig. 6.

Correlation of S.E (18 drugs) and F_s (present study) for all 14 columns. The S.E. (18 drugs) is the standard error of $\log k'$ plot for the 18 drugs on the test column against the Discovery C18 column. ($S.E._{18 \text{ drugs}} = 0.0026 (\pm 0.0002) F_{s, \text{ present study}} + 0.008 (\pm 0.016)$; $r^2 = 0.943$; $S.E. = 0.04$). Other conditions as stated in Fig. 1.

Fig. 7. Plot of S.E. (18 drugs) vs. S.E. (S-D solutes) for all 14 columns. ($S.E._{18 \text{ drugs}} = 0.727 (\pm 0.07)$ $S.E._{S-D \text{ solutes}} + 0.02 (\pm 0.02)$, $r^2 = 0.898$; S.E. = 0.06). All other conditions as stated in Fig. 1.

Fig. 8. Panels (A) and (C) – score plots; panels (B) and (D) - dendrograms based on Euclidean distances. The dashed vertical lines show which groupings exist at the distance given on the x-axis; panels (A) and (B) - data for S-D solutes; Panels (C) and (D) - data for drug solutes. All plots based on analysis of double mean-centered log k' data.

Fig. 9.

Relationship between similarities based on distances for the drug and S-D solutes. The circled data point corresponds to the ZirChrom PS/HC-SO₃⁻ column comparison. ($D_{\text{drugs}} = 0.78 (\pm 0.05) D_{\text{S-D}} + 0.16 (\pm 0.09)$; $r^2 = 0.737$; S.E. = 0.43)

Table 1

Columns used in the present study

Column Group	Columns	Type	F_s^a	$F_s'^b$
I (Very similar)	Discovery C18 - Supelco	endcapped	0.0	0.0
	ACE 5 C18 - Mac Mod	endcapped	3.3	3.3
	Alltima HP C18 - Alltech	endcapped	9.8	9.8
	Zorbax SB C18 - Agilent	bulky silane	13.6	13.6
II (Similar)	Beta Basic Phenyl – Thermo	endcapped	22.8	0.0
	Zorbax Phenyl - Agilent	endcapped	23.9	15.5
	Prontosil 60-5-Phenyl - Bischoff	endcapped	30.0	29.7
III (Similar)	Hypersil Prism RP - Thermo	polar embedded/EPG	252.3	0.0
	Bonus RP – Agilent	polar embedded bulky silane/EPG	268.0	24.1
IV (Very different)	HC-OH - Carr laboratory ^c	hypercrosslinked phase	23.7	0.0
	Nova Pak CN HP-60 – Waters	endcapped	48.0	57.3
	ZirChrom PS ^d –ZirChrom	polystyrene-coated zirconia	133.9	136.9
	HC-SO ₃ ⁻ - Carr laboratory ^c	hypercrosslinked phase	205.2	207.8
	Inertsil CN-3 – GL Science	non-endcapped	232.3	235.2

^a F_s values of a given column vs. the Discovery C18 column based on S-D column parameters.

^b F_s' is F_s computed relative to a reference column in each group. $F_s' = 0$ denotes the reference column.

^c Ref. [50, 51, 54]

^d A column packed with polystyrene-coated zirconia particles. It is an ideal reversed-phase for highly aqueous eluents.

Table 2

Fitted value of the column parameters based on measurement of 16 standard solutes

Column Group	Columns	Column size (mm)/pore size (Å)	V_m^a	H^b	S^*b	A^b	B^b	C^b	$\log k'_{(ob)}^c$
Group I	Zorbax SB C18	50 × 4.6/80	0.439	0.980	-0.032	0.305	minus;0.003	0.107	0.918
	ACE 5 C18	50 × 4.6/100	0.515	0.986	0.035	0.035	minus;0.007	0.086	0.873
	Discovery C18	50 × 4.6/180	0.468	1.031	0.060	0.080	minus;0.027	0.018	0.972
	Alltima HP C18	50 × 4.6/190	0.556	0.980	0.032	0.028	0.001	0.159	0.711
Group II	Zorbax SB Phenyl	50 × 4.6/80	0.482	0.612	minus;0.161	0.070	0.036	0.088	0.498
	BetaBasic Phenyl	50 × 4.6/150	0.630	0.574	minus;0.152	minus;0.129	0.071	0.149	0.253
	Prontosil 60-5 Phenyl	50 × 4.6/60	0.477	0.660	-0.149	-0.064	0.001	0.113	0.671
Group III	Hypersil Prism RP	50 × 4.6/100	0.516	0.659	0.012	-0.161	0.287	-1.281	0.703
	Bonus RP	50 × 4.6/80	0.432	0.760	0.012	-0.096	0.309	-1.334	0.738
Group IV	Inertsil CN-3	50 × 4.6/100	0.565	0.349	-0.002	-0.491	0.088	-0.954	0.077
	Nova Pak CN HP-60	50 × 4.6/60	0.541	0.237	-0.118	-0.069	0.034	0.524	-0.362
	ZirChrom PS	150 × 4.6/300	1.564	0.778	-0.280	-0.928	0.135	1.903	-0.571
	HC-OH	50 × 4.6/80	0.602	0.350	-0.368	-0.237	0.138	0.142	-0.120
	HC-SO ₃ ⁻	50 × 4.6/80	0.492	0.542	-0.508	0.063	0.104	2.164	0.371

^a V_m is the dead volume in mL

^bValue obtained in this study by least squares fit of $\log k'$ vs. S-D solute parameters (η' , σ' , β' , α' , κ') based on our measured value of solutes k' relative to uracil. Chromatographic conditions: 50% ACN/buffer (30 mM, pH 2.8), 35 °C; 1.0 mL/min.

^c $\log k'$ of ethylbenzene

Table 3

The differences between our measured column parameters and S-D column parameters

Column Group	Columns	ΔH^a	ΔS^a	ΔA^a	ΔB^a	ΔC^a	S.E. ^b (measured)	S.E. ^c (S-D)	Ref. ^e
Group I	Zorbax SB C18	-0.018	0.000	0.030	-0.004	-0.034	0.010	0.003	[15]
	ACE5 C18	-0.014	0.008	0.130	0.000	-0.058	0.005	0.001	[15]
	Discovery C18	0.047	0.033	0.208	-0.031	-0.158	0.009	0.003	[15]
	Alltima HP C18	-0.013	0.047	-0.008	0.014	0.067	0.006	0.005	[15]
Group II	Zorbax SB Phenyl	-0.011	0.000	0.005	-0.002	0.056	0.017	<i>d</i>	
	BetaBasic Phenyl	-0.008	0.006	0.282	0.022	0.045	0.050	0.032	[19]
	Prontosil 60-5 Phenyl	-0.045	0.045	-0.061	0.011	-0.298	0.032	0.037	[19]
Group III	Hypersil Prism RP	0.014	-0.077	0.298	-0.015	1.536	0.114	0.110	[17]
	Bonus RP	0.106	-0.095	0.950	-0.064	1.637	0.134	0.140	[17]
Group IV	Inertsil CN-3	-0.020	-0.051	0.317	0.005	1.653	0.047	0.055	[18]
	Nova Pak CN HP-60	-0.125	0.047	-0.169	0.034	-0.168	0.109	0.020	[18]
	ZirChrom PS	0.189	-0.047	-0.451	0.073	0.153	0.124	0.110	[16]
	HC-OH	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	0.112	<i>d</i>	
	HC-SO ₃ ⁻	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	<i>d</i>	0.088	<i>d</i>	

^aDifferences between our measured column parameters and S-D column parameters.

^bStandard error of fit of our measured values of log k' and measured H, S*, etc. to Eq. (1).

^cStandard error reported by S-D for their measured values of log k' to Eq. (1).

^dNot measured by S-D.

^eReference for S.E. from S-D work.

Table 4
Standard errors for column pairings based on 18 drugs under isocratic elution conditions

Columns	Discovery C18	ACE C18	Alltima C18	SB C18	BetaBasic Phenyl	Prontosil Phenyl	Zorbax Phenyl	Nova Pak CN	Inertsil CN	HC- OH	Prism RP	Bonus RP	ZirChrom PS
ACE 5 18	0.01												
Alltima HP C18	0.01	0.01											
Zorbax SB C18	0.05	0.04	0.05										
BetaBasic Phenyl	0.04	0.04	0.04	0.05									
Prontosil 60-5 Phenyl	0.07	0.06	0.07	0.05	0.07								
Zorbax Phenyl	0.11	0.10	0.11	0.08	0.09	0.06							
Nova Pak CN	0.15	0.15	0.15	0.16	0.16	0.16	0.16						
Inertsil CN	0.19	0.18	0.17	0.17	0.20	0.16	0.15	0.17					
HC-OH	0.25	0.25	0.25	0.25	0.24	0.25	0.27	0.31	0.28				
Prism RP	0.27	0.26	0.27	0.24	0.29	0.22	0.22	0.16	0.08	0.28			
Bonus RP	0.34	0.33	0.34	0.30	0.37	0.28	0.27	0.17	0.13	0.28	0.06		
ZirChrom PS	0.42	0.41	0.41	0.43	0.42	0.41	0.42	0.57	0.48	0.30	0.48	0.48	
HC-SO ₃ ⁻	0.52	0.52	0.52	0.52	0.52	0.51	0.51	0.49	0.42	0.52	0.43	0.42	0.52

Table 5

Standard errors and % cumulative variance for S-D and drug solutes

Number of Components	Standard Errors		% Cumulative Variance	
	S-D Solutes	Drugs	S-D Solutes	Drugs
1	0.151	0.163	79.54	65.95
2	0.082	0.119	94.84	84.27
3	0.063	0.095	97.44	91.51
4	0.044	0.060	98.95	97.15
5	0.030	0.044	99.59	98.70