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Validation Method Used In Quantitative Structure Activity Relationship

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ABSTRACT

Quantitative structure activity relationship is study of relationship between physiochemical (independent) properties and biological (dependent) activity of bioactive molecules. It plays an important role in the drug discovery and development and involves steps including data preparation, data analysis, and model validation. Validation is important process in QSAR analysis. After correlation between, independent and dependent variable with the help of various statistical methods the model will developed now that model should be validated. Various types of statistical methods are used in QSAR analysis such as principle component analysis, cluster analysis, simple linear regression, multiple linear regressions, partial least square, K-Nearest Neighbor classification, neural network, logistic regression and many others. After the development of model it is necessary to find out how predictive a model is that is concept of validation, which finds out the accuracy of model to predict the activity of bioactive compound. Validation is important in QSAR analysis since it is not necessary that a good model always have a good ability to predict the activity of respective bioactive agents. Validation involves external and internal validation to predict external and internal predictivity respectively. Cross validation (K-fold cross-validation, leave-one-out cross-validation, leave-ten-out or leave-many-out cross-validation), bootstrap method, randomization, jack- knife, hold out validation methods are used for internal validation to predict the internal validity. In the external validation data selection plays an important role because the distribution of the properties (variables) should be uniform in both of the sets that are test set and training set. There are various method through which the original data can accurately divided involving manual selection, randomization, sphere exclusion method and other methods such as onion design, cluster analysis, factorial, full factorial experiment, self organizing map(SOM), and principle component analysis. With the help of various statistical measures such as n, k, df, r^2, q^2, pred_ r^2 etc. and there recommended values we can easily validate the model.

Keywords: Validation, QSAR, Statistical methods.

INTRODUCTION

Quantitative structure activity relationship (QSAR) is method in which structural, chemical, physical and other properties of a compound correlate to his biological activity. These physiochemical properties include topology, electrical properties, steric effects as well as others.
QSAR related studies starts over 100 years ago. In 1863, A. F. A. Cros at the University of Strausbourg that alcohol toxicity was inversely correlated with the solubility of the alcohol in water. In 1899, H. H. Meyer published a paper that described that the toxicity of organic compounds depended on their lipophilicity. These facts proofed a relationship between solvent portioning and biological properties.

Now a day in modern QSAR (also termed as 2D or 3D-QASR), QSAR relations are determined by using statistical correlation of structural descriptor and biological activity. Physiochemical properties are correlated to biological activity using statistical method. Most popular and commonly used 3D-QSAR involved model computational method and technique such as 3D-visualization, statistical correlations, statistical validation many other improved technique to predict the activity of calculated compound [1-2].

Quantitative structure activity relationship has been applied extensively and successfully over several decades to find predictive model for activity of bioactive agent. It has been used in several areas that is related to drug discovery and development, predict the activity of several therapeutic agents, physiochemical properties prediction [3], ADME studies [4-5], drug resistance [6], drug metabolism [7], toxicity prediction [8-9] and other several areas.

In the QSAR studies involves steps data preparation [1-2] which deals with selection of appropriate data set that is used in study, and data analysis [1-2] which deals with selection of appropriate technique for statistical analysis and correlation studies. Now by using these statistical analysis and correlation studies we develop a model, after the development of a model with the use of various data and statistical methods it is necessary to find out how predictive a model is, from here the concept of validation starts which find out the accuracy of the model to predict the activity of bioactive agent.

VALIDATION

QSAR studies aimed to derive a model that is optimally active, means the model should provide a reliable estimate of the activity of new or untested compound similar to those in it. After development of the model it is necessary to test whether any data from the data set affect model extensively. This is done by using QSAR validation methods. Validation method is required to ensure model reliability, to ensure that the model is not due to chance factor. Various types of validation methods are used in the QSAR studies to predict the accuracy of model or to estimate the validity or predictivity of the derived structure property model.

Validation Methods

Internal validation

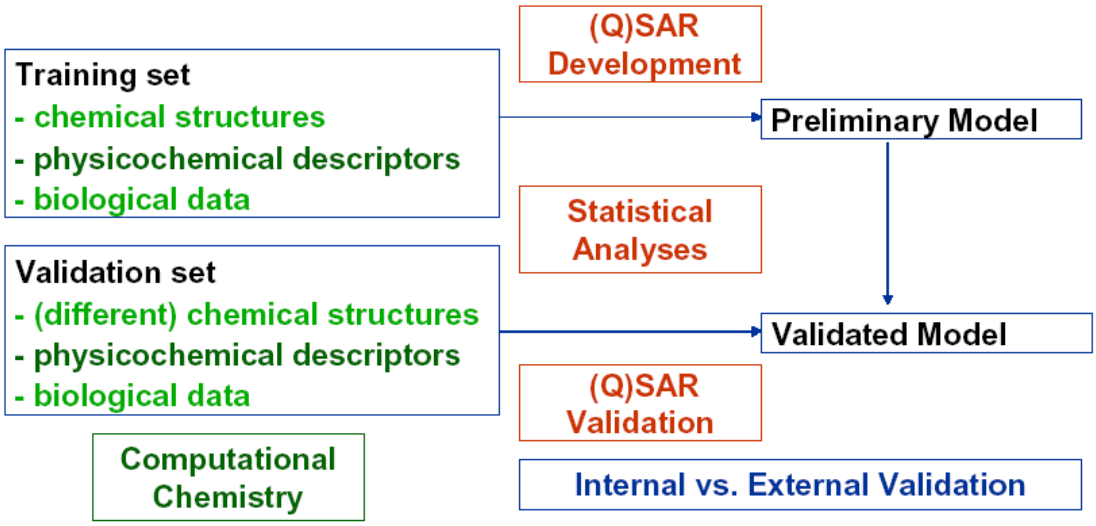
In this type of validation we use a dataset from which the model is derived and check the internal consistency. A new model develops by using a reduced set of data. This new model is used to predict the activity of the compound that is not included in the new model data set. This is

repeated until all compounds have been deleted and predicted ones. This method is less critical than external validation.

External validation

In this validation the original data are divided into two sets that are training set and test set. The training and test set can be divided by several approaches (ref- report on the workshop on the validation of QSAR). One of approach is that a fixed proportion of a homogeneous data set from the original data set is omitted, thereby by forming a test set, and remaining a training set. If the training set contain two third of original data and test set one third, then the data could be rank-ordered according to the magnitude of the biological response. Other approaches can also be used to the selection of training set such as on the basis of relevant physiochemical properties [11]. Training set is used to derive a model, and this model is used to predict the activity of test set members.

Figure 1: Development and validation of QSARs



Validation method commonly used in QSAR studies

Randomization test

Randomization test or Y-scrambling is important popular mean of statistical validation. In this the output descriptors such as biological response of the compound are scrambled tougher, and these scrambled date set are re-examine against unscrambled real input descriptors to determined the predictivity and correlation of the resulting model. Entire procedure it repeated Multiple (typically 20-50 models) times on many differently scrambled data sets. If there is a strong correlation selected variables and randomized responses variables, then significance of the proposed QSAR model is regarded as suspect. During the randomization test following values are calculated and recorded.

- Number of randomization trials.
- Value of R from the non-random trial.
- Total number of R values from random trail that are less than R value of non random trail.

- Total number of R values from random trail that are greater than R value of non random trail.
- Used confidence level.
- Mean value of R for all random trails.
- Standard deviation of R value of all random trails from the mean value of R.
- The number of standard deviation of mean value of R of all random trials to the non random R value. The larger this number greater the probability that the model generate with nonrandom data represent a true relationship between the data variables and activity.

Cross validation

The theory of cross-validation was inaugurated by Seymour Geisser. It is most popular method for the determination of prediction accuracy. Cross validation also called rotation estimation [12-14] is statistical practices, which involves partition of sample or main data in subsets and initially perform the analysis on a single subset. The other remaining subset are retain for subsequent use in conforming and validating the initial analysis. Sub set of data that is used for initial analysis is called training set and other data that used for conforming and validating the initial analysis is called validation or test set.

Cross-validation is important statistical validation technique that avoids over-fitting models on training data, as over-fitting will give low accuracy on validation. It also helps to right set of descriptors, an appropriate algorithm and associated parameter for a given dataset [15].

Common types of cross-validation:-

- Jack-knife
- Hold out validation
- K-fold cross-validation
- Leave-one-out cross-validation
- Bootstrap analysis

Jack-knife

This technique is also based on data splitting. Here the original data set is randomly split in two subsets. First set is called training set of compound that is used for exploration and model development, and other set is used for prediction and validation of model, called test set or validation set.

It corresponds to leave one out cross-validation; used for the estimation of confidence intervals of nonlinear parameters, like ß, and log Po [16].

In the cross validation leave-one-out cross-validation is easily confuse with jack-knifing. Both methods involved splitting of main data set in to training set and test set. Both involve omitting each training case in turn and retraining the network on the remaining subset. But main difference is that the jack-knife method is used to estimate the bias of a statistic while leave-one-out cross-validation is used to estimate the generalization error.

In the jackknife, you compute some statistic of interest in each subset of the data. The average of these subset statistics is compared with the corresponding statistic computed from the entire

sample in order to estimate the bias of the latter. You can also get a jackknife estimate of the standard error of a statistic. Jackknifing can be used to estimate the bias of the training error and hence to estimate the generalization error, but this process is more complicated than leave-one-out cross-validation [17].

Hold out validation

It also known as split sample method. In a common sense holdout validation is not a cross-validation because in this type of method the data never crossed over. In this validation the validation data is formed by randomly choosing the observations from the initial data and remaining data is used as training set. Normally less than a third of initial sample is used for validation data [18].

The holdout estimate is a random number that depends on the division into a training set and a test set. In random sub sampling the holdout method is repeated *k* times and the estimated accuracy is derived by averaging the runs. The Standard deviation can be estimated as the standard deviation of the accuracy estimations from each holdout run [14].

In the split-sample method, only a single subset that is validation set is used to estimate the generalization error, instead of k different subsets; i.e. there is no "crossing".

K-fold cross-validation

In the K-fold cross-validation we divide the original sample data set in to K-subset of approximately equal size. Out of K-subsets a single sub sample is retain as validation data for testing the model, and remaining K – 1 subsamples is used as training data. The cross-validation process is then repeated *K* times, with each of the *K* subsamples used exactly once as the validation data. The K results from the folds then can be averaged to produce a single estimation.

Leave-one-out cross-validation

In the Leave-one-out cross-validation we use only a single observation from the original sample data as a validation data and the remaining obervation is used as training data. This process is repeated such that each obervation in the sample is used once as a validation data. The remaining training data is used to constructs a model that is subsequenty used to predict the removed sample or validation data. This procedure is repeated for all data points so that a complete set of predicted value can be obtain [19-20].

After cross-validation, the cross-validated correlation coefficient (*q*2) that resulted in optimum number of components and lowest standard error of prediction were calculated using following formulae.

$$q^2 = 1 - \frac{\sum_y (y_{pred} - y_{observed})^2}{\sum_y (y_{observed} - y_{mean})^2}$$

$$PRESS = \sum_y (y_{predicted} - y_{observed})^2$$

Where, y_{pred}, y_{actual} and y_{mean} are predicted, actual, and mean values of the target property (biological activity) respectively. The predictive power of QSAR models, derived by using the

training set were examined by an external test set of eleven molecules. The predictive ability of the models is expressed by the predictive r^2 value, which is analogous to cross-validated r^2 (q^2) and is calculated using the formula:

$$r^2_{pred} = \frac{SD - PRESS}{SD}$$

Where, SD is the sum of the squared deviations between the biological activities of the test set and mean activities of the training molecules and PRESS is the sum of squared deviation between predicted and actual activities of the test set molecules [21].

Bootstrap analysis

Bootstrapping is another type of re-sampling method that is different from data splitting. It is statistical simulation method which generates sample distribution from the original data set [22]. Bootstrapping seems to work better than cross-validation in many cases [23]. In the simplest form of bootstrapping, instead of repeatedly analyzing subsets of the data, you repeatedly analyze sub-samples of the data. Each sub-sample is a random sample with replacement from the full sample. The concept of bootstrapping is founded on the premise that the sample represents an estimate of the entire population, and that statistical inference can be drawn from a large number of pseudo-samples to estimate the bias, standard error, and confidence intervals of the parameter of interest. The pseudo- (or bootstrap-) samples are created from the original data set by sampling with replacement, where some objects may appear in multiple instances. The usual point of contention about the bootstrap procedure concerns the minimal number of samplings required for computing reliable statistics. An empirical rule given by Davison and Hinkley suggests that the number of bootstrap-samples should be at least 40 times the number of sample objects [24].

External Validation

It is important part of the validation study, it performs to ensure that model have acceptable predictive power. One of the important steps in the external validation is division of original data set in to training and test set data or we can say selection of training and test set.

Selection of training and test set

QSAR models are used increasingly to screen chemical database and/or virtual chemical libraries for potentially bioactive molecules. These development emphasize the importance of rigorous model validation to insure that the model have both the ability to explain variance in the biological activity (internal validation) and also the acceptable predictive power (external validation).

For model validation original dataset is required to divided in to training set and test set, here training set is required to build a model and test set is required for examine the predictive ability of model. For any QSAR model it is of crucial importance that the training set selected to calibrate the model exhibits a well balanced distribution and contains representative molecules.

Following are some of the method that are commonly used for division of the original dataset in to training and test set:

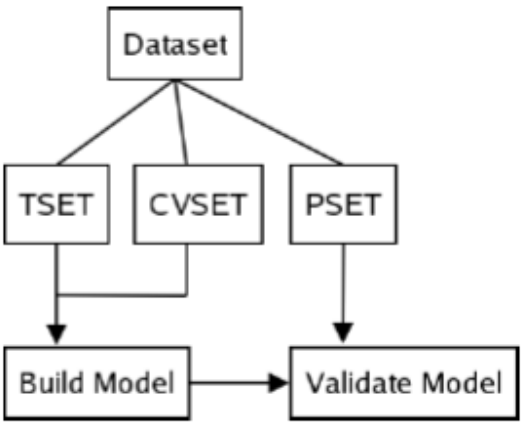
1) **Manual Selection:**
In this method we divide the dataset in to test set and training set by manually visualizing the variation in the chemical and biological properties in the given dataset.

2) **Random selection:**
In this method the dataset is divided in to training and test set by random distribution. The points in the given dataset are selected randomly, but most important thing is it should be uniformly distributed in both the sets.

3) **Sphere Exclusion method:**
This is another method which is used for division of the dataset in to test and training set. In this method we use probe spheres to set a similarity limit. A radius of the sphere is given by equation.

$$R = c\left(\frac{V}{N}\right)^{1/K}$$

Depend on a user defined constant, c, called the Disimilarity Level. In this method first we select compound with highest activity add this to the TSET, than constrict sphere centered at this point (compound with highest activity) having radius R. The entire compounds within the sphere go in to the TSET. Exclude the point selected from the data set. Calculate distance between all remaining compound and constructed sphere centers. Generate the TSET and PEST, however it is difficult to exactly get a TEST specified size as a result we need to vary c by trail and error. Ones we have TSET we randomly select a CVSET from it. In this way generate test and training set. Sphere exclusion method ensure that the point in the both the sets are uniformly distributed with respect to chemical and biological activity [25].



4) **Other methods:**
   a) Experimental Design: full factorial, fractional factorial etc.
   b) Onion Design:

c) Cluster Analysis:
d) Principle Component Analysis:
e) Self Organizing Maps(SOM)

a) Experimental Design: full factorial, fractional factorial

Original data can also be divided by using experimental design such as full factorial, or fractional factorial design. These two experimental Designs provide an important mean for the data division in the QSAR studies.

Full factorial:

A full factorial design is type of design in which every setting of every factor appears with every setting of every other factor. Full factorial designs not recommended for 5 or more factors. Following types of full factorial design can commonly used.

●Full factorial designs in two levels

A common experimental design is one with all input factors set at two levels each. These levels are called `high' and `low' or `+1' and `-1', respectively. A design with all possible high/low combinations of all the input factors is called a full factorial design in two levels. If there are k factors, each at 2 levels, a full factorial design has 2^k runs.

●Full factorial designs in three levels

The three-level design is written as a 3^k factorial design. It means that *k* factors are considered, each at 3 levels. These are (usually) referred to as low, intermediate and high levels. These levels are numerically expressed as 0, 1, and 2. One could have considered the digits -1, 0, and +1, but this may be confusing with respect to the 2-level designs since 0 is reserved for center points. Therefore, we will use the 0, 1, 2 scheme. The reason that the three-level designs were proposed is to model possible curvature in the response function and to handle the case of nominal factors at 3 levels. A third level for a continuous factor facilitates investigation of a quadratic relationship between the response and each of the factors.

●Fractional factorial

fractional factorial designs are experimental designs consisting of a carefully chosen subset (fraction) of the experimental runs of a full factorial design. The subset is chosen so as to exploit the sparsity-of-effects principle to expose information about the most important features of the problem studied, while using a fraction of the effort of a full factorial design in terms of experimental runs and resources. Fractional designs are expressed using the notation l^{k-p}, where *l* is the number of levels of each factor investigated, *k* is the number of factors investigated, and *p* describes the size of the fraction of the full factorial used. Formally, *p* is the number of generators [26].

b) Onion design

It is another method that can use for division of dateset in to training and test set. It is a type of design which combine the best properties of other design families have model supporting ability of D-optimal design and the uniform coverage ability of space filling design [27-28]. In this type of design the candidate set or original data set are split in to number of sub-sets that are known as ‘shells’ or ‘layers’ and a D-optimal selection is

made from each shell. This makes it posible to select representative sets of molecular structure throughout any property space with resonable design size. The number of selected molecules is easily controlled by varying (i) the number of shells and (ii) the model on which the design is based [28]. There are a number of adjustable parameter which define the shape and scope of D-optimal onion design, making the approach extremely flexible. One important cosideration is the number of PCA-or PLS-components underlying the design. The user must define the number of layer, the thickness of the layer, the number of runs, and the type of regression model used within each layer.

c) Cluster Analysis

It is another important tool for the division of dataset in to sub-sets. Cluster analysis is method of classification of object in to different groups or partitioning of a data in to subsets or clusters where the members of subsets or groups share properties in common. Data clustering is a common technique for statistical data analysis, which is used in many fields, including data division in QSAR studies. Data clustering algorithms can be hierarchical or partitional. An important step in any clustering is to select a distance measure, which will determine how the *similarity* of two elements is calculated. This will influence the shape of the clusters.

d) Principle component analysis

Principle component analysis is statistical method that is used to multidimensional data sets to lower dimension data sets for analysis. It involves a mathematical procedure that transforms a number of (possibly) correlated variables into a (smaller) number of uncorrelated variables. It is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. PCA is mathematically defined as an orthogonal linear transformation that transforms the data to a new coordinate system such that the greatest variance by any projection of the data comes to lie on the first coordinate (called the first principal component), the second greatest variance on the second coordinate, and so on. PCA is theoretically the optimum transform for a given data in least square terms.

e) Self Organizing Maps (SOM)

Self-organizing maps (SOMs) are a data visualization technique reduces the dimensions of data through the use of self-organizing neural networks. The problem that data visualization attempts to solve is that humans simply cannot visualize high dimensional data as is so techniques are created to help us understand this high dimensional data. The way SOMs go about reducing dimensions is by producing a map of usually 1 or 2 dimensions which plot the similarities of the data by grouping similar data items together. So SOMs accomplish two things, they reduce dimensions and display similarities.

Statistical methods used in QSAR analysis

Statistical methods are an essential component of QSAR work. They help to build models, estimate a model's predictive abilities, and find relationships and correlations among variables and activities. A suitable statistical method coupled with a variable selection method allows

analysis of this data in order to establish a QSAR model with the subset of descriptors that are most statistically significant in determining the biological activity. The statistical method can be broadly divided in to two: linear and non-linear method. In statistics a correlation is established between dependent variables (biological activity) and independent variables (physiochemical properties or molecular descriptor). The liner method fits a line between the selected descriptor and activity as compared to non-linear method which fit a curved between the selected descriptor and activity.

The statistical method to build QSAR model is decided based on the type of biological activity data.

Following are commonly used statistical methods

1. Principal component analysis (PCA)
2. Cluster analysis
3. Simple liner regression
4. Multiple liner regression
5. Stepwise multiple liner regression
6. Principle component regression (PCR)
7. Continuum Regression
8. Partial least squares (PLS)
9. Genetic function approximation (GFA)
10. Genetic partial least squares (GPLS)
11. Logistic regression
12. K-Nearest Neighbor classification (KNN)
13. Neural Network
14. Discriminant analysis
15. Decision Trees
16. SIMCA
17. Canonical Correlation

Evaluation of the model

It is important part of QSAR analysis after development of the model it is important to evaluate the significance of the model. There are various statistical measures available that are used for the evaluation of the model, followings are most commonly used:

Statistical measures	Minimum recommended values
N	Number of molecules (>20 molecules)
K	Number of descriptor in a model (Statistically n/5 descriptor in a model.
Df	Degree of freedom (n-k-1) (higher is better)
r^2	Coefficient of determination (>0.7)
q^2	Cross-validated r^2 (>0.5)
pred_r^2	r^2 for external test set (>0.5)
SEE	Standard error of estimate (smaller is better)

F-test	F-test for statistical significance of the model (higher is better, for same set of descriptor and compound)
F_prob.	Alpha error probability (smaller is better)
Z score	Z score calculated by the randomization test (higher is better)
best_ran_q^2	Highest q^2 value in the randomization test (as low as compared to q^2)
best_ran_r^2	Highest r^2 value in the randomization test (as low as compared to r^2)
Alpha	Statistical significance parameter by randomization test

CONCLUSION

The challenge in QSAR studies is not only constructing a model that is statistically able to predict the activity within the training set but also developing a model with the capacity to accurately predict the activity of untested chemical [10]. When fitting any sort of predictive model it is essential to verify that the fitted model can be generalized to future data of the same type. The model validation is essential and critical but often neglected component of QSAR development [29]. Not only proper training but also proper validation process is required in QSAR analysis. The present review is aimed to conclude various method of validation used in QSAR analysis and their importance to develop an effective and better accurate QSAR models.

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