

Method Validation in a PAT Environment using a SAS® Enterprise Guide Add-In

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ABSTRACT

In this paper analytical method validation with the aid of SAS® Enterprise Guide add-ins is discussed. Examples are presented of add-ins that address assay validation issues in a PAT (Process Analytical Technology) environment. Streamlining method validation, robustness tests with experimental designs, multivariate analysis, real-time data and control charts are topics that will be demonstrated in these applications.

INTRODUCTION

This paper describes a number of small applications that attempt to streamline and simplify the validation of assay methods in a PAT environment. Descriptions of developments concerning the PAT initiative can be found in the literature (1,2,3).

Although the ultimate goal of the applications is to assist in the registration of specific drugs, these initial versions will have an open, prototype character. SAS Enterprise Guide is well suited for the further specification and modification of the applications to a form usable in a compliant pharmaceutical environment.

The key objective is to be able to address diverse situations in a PAT environment with a number of relevant applications available in a prototype version,. Another objective is the streamlining and eventually the automation of the validation (4). The applications presented in this paper are being developed from an Analytical Chemistry perspective and are based on current examples from the literature, in particular from liquid chromatography.

PROTOTYPE I (METHOD VALIDATION)

The first prototype application is based on a document from the Dutch Food Authorities, 'Validatie van methoden' (5). Although this document is not intended for a pharmaceutical environment, it is based on a number of international standards and well delimited. Not only is this document not based on pharmaceutical standards, it is also outdated and must be adapted on a number of points. With a number of additional, prototype applications an attempt will be made to link to a contemporary PAT environment.

A flow diagram from the document is contained in the application. The validation of assay methods consists of a number of familiar elements that must be completed in a certain order. A number of selections in the beginning of the application confine the further course. The familiar elements are the analysis of:

- the selectivity/specificity
- the signal-to-noise ratio
- the calibration curve
- the limit of detection/quantitation
- the trueness /recovery
- the precision
 - the repeatability
 - the reproducibility

To keep the application as simple as possible calculated values are stored in temporary datasets. The user points at a dataset of measurement results, calculates a number of parameters and reports on the results.

In a regulated PAT environment this prototype will need a number of technical modifications and content adjustments.

Some necessary content adjustments are:

1. The robustness.

An analytical procedure is considered robust when the precision and trueness (accuracy) of the method are insensitive to minor changes in environmental and procedural variables (IUPAC). According to ICH guidelines, robustness tests are not obligatory yet. However, they are demanded by the FDA for the registration of drugs in the USA. Robustness is considered an essential stage in the validation of a procedure and should be verified at the end of the method development or at the beginning of the validation (6,7,8). Such a robustness test should study the effect of the experimental factors intrinsic to the method such as temperature, changes in composition of the mobile

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phase, the reaction time, etc.

Robust statistical evaluation should be able to deal with the unavoidable presence of anomalous data. Statistical tools that do not require any particular distribution and are resistant to outlier data are preferred and usually based on the median which, in general, provides a better idea of the central trend than the mean of the data itself (8,9).

2. The selection of the linear range of the calibration curve.

Calibration is an important step in the chemical analysis. Linear calibration curves are preferred because they are simple to construct, evaluate and control (8,9). Transformations and formal assessment of the linear range can facilitate this step in the construction and validation of the calibration line.

3. The analysis of the selectivity/specificity

The standard addition method is an efficient way to study the selectivity/specificity. By comparing the slopes of the standard addition line and the aqueous calibration line matrix interferences can be traced (8). This selectivity can possibly be enhanced by using modern spectroscopic analytical techniques and multivariate analysis (10).

4. The use and analysis of real-time data.

In the PAT initiative the use of real-time data is encouraged (1).

Some of these necessary adjustments are executed in the form of prototype applications with additional functionality.

PROTOTYPE II (ROBUSTNESS)

The first added functionality addresses the robustness of the assay method. In a robustness test the influences of small deliberate changes in "procedure related" method parameters, such as operational, environmental and peak measurement/analysis parameters are evaluated (USP/ICH) (6). Usually an experimental design approach with a Plackett-Burman or fractional factorial screening design is chosen for this robustness test to minimize the number of experiments. Although this experimental design approach could be used to study interactions between factors, they are usually considered negligible. Asymmetrical factorial designs are an alternative to consider non-procedure-related factors for chromatographic methods, such as the chromatographic column manufacturer, where more than two levels should preferably be studied.

A proper reporting of robustness testing should include the selected factors, and their levels, a specification of the experimental design used, the responses considered, the planning and execution of the experimental work, and finally the analysis of the results (graphically and/or statistically) (11).

This prototype is modeled on an article from van der Heyden et al. and like the first application the flow diagram is contained in the application (Fig. 1) (11).

In the experimental design procedure different steps can be distinguished:

1. Selection of factors and their levels
2. Selection of an experimental design
3. Selection of responses
4. Planning and execution of the experimental set-up and measuring the responses.
5. Calculation of the factor effects on the responses
6. Graphical and/or statistical interpretation of the estimated effects
7. Making chemically relevant conclusions
8. Defining system suitability test limits for certain qualitative responses.

The prototype is developed with an Oracle Express database in the background, and thus closer to an ultimate version for use in a regulated pharmaceutical environment. The user selects a number of factors, responses and a design. The experimental and calculated values are stored in the Oracle database and can thus be compared easily (Fig. 2). Estimated effects are graphically and statistically interpreted to determine their significance as recommended. Graphical methods consist of drawing normal probability plots and bar graphs (Fig. 3). In the normal probability plots the non-significant effects are found on a straight line, while the significant effects deviate from this line, based on the fact that if the variation in the data is due solely to a random variation and the changes in level of the factors have no effect on the response, then the coefficients will have a normal distribution and will seem to be aligned. Bar graphs contain a bar for each effect. The bar lengths are equal to the standardized effects of each factor, which are in fact the t-values.

Statistical significance of the effects was determined by comparing each calculated effect with a critical value derived from a t-test. The standard error of an effect (SE_e) can be determined in different ways. An estimate of the experimental error (SE_e) can be calculated for each response from the three dummy factors available in the Plackett-Burman design. The dummies do not represent anything physical or chemical and thus their occurrence at low and high level has no impact on the execution of the analysis protocol. However, the effects calculated for these dummies, which are due to experimental error, can be used in the statistical interpretation of the factor effects on the different experimental responses (12).

In another approach, (SE_e) is derived from a posteriori defined negligible effects by using the algorithms of Lenth or Dong. The advantage of both methods is the use of the median which is a more robust estimator. The algorithm of

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Dong is said to lead to practically more relevant E_{critical} values (8,11).

An SST (System Suitability Test) is often performed to evaluate the performance of an analytical method. Examples are peak resolution, peak asymmetry, analysis time, etc. These qualitative factors or SST responses can be examined and the observed influence on the SST responses can then be used to define SST limits. Certain qualitative responses should be within given SST limits.

ICH guidelines recommend SST limits to be defined based on the results of a robustness test. When the quantitative aspect of the method is considered robust, the experimental conditions showing the worst result for the SST response are derived from the results of the applied experimental design (worst case conditions) (11).

POSSIBLE EXTENSIONS

A possible extra functionality could be one that determines the linear range of the calibration curve in a formal, robust way (8). Usually the least squares (LS) method, which minimizes the sum of the squares residuals is used, which assumes that the errors are with normal distribution, with constant variance, and that they are independent of each other and of the concentration level. However, this least-squares method is very sensitive to the presence of outliers. Consequently it is not useful for determining the linear range of a calibration curve because any curvature in the response behaves in the same way as outliers. The lack of normality in the probability distribution caused by the outliers affects not only the evaluation of the slope and the intercept, but also the precision of the estimations. A more robust regression technique, the least median of squares (LMS) regression which minimizes the median of the squared residues, can be employed for the determination of those points which are outside the linearity, followed by LS method to the aligned experimental points (8,9). This functionality has not been added yet.

In a usual univariate analysis physical and chemical treatments are required to eliminate interferences. With multivariate analysis one can attempt to eliminate interferences by means of mathematics. There is a large interest in multivariate calibration because the analytical procedure is fast, cheap and accurate enough for many real problems (10). It is usually applied to complex real matrices, where the separation procedures and chemical treatments necessary to apply the usual simple calibration (based on one specific predictor) are expensive and time consuming. The interesting chemical or physical quantity (response) is obtained as a function of many measured quantities (predictors). Multivariate calibration uses non-specific predictors, generally physical information from spectra. The function that computes the response from predictors is obtained by means of chemometric tools, able to extract a specific model from many non-specific predictors. In samples the unknown value of the response can be estimated using the regression model. The prediction error is a measure of the error of this estimate. The procedure used to evaluate the prediction error is known as validation (10).

Another possible prototype application could be one that assists in this multivariate analysis. In a flow diagram according to PASG (Pharmaceutical Analytical Sciences Group) a distinction is made between MLR (Multiple Linear Regression) en Multivariate Analysis with correlation (13). A MLR application is probably a relatively simple extension of the first prototype application with a number of linear calibration lines instead of just a single one. However, with MVC (Multivariate Calibration) things are more complicated. On the one hand there is code available for the calculations; on the other hand one has to deal with numerous options in these calculations (14, 15). Without close cooperation with the user this is not easily contained in a flow diagram. This software is often part of the instrument, and the data treatment assumes a faith in the instrument (10).

PROTOTYPE III (MULTIVARIATE CONTROL)

To get acquainted with MV analysis and build a relevant application, the following imaginary situation was contemplated. EBA (Expanded Bed Adsorption) process chromatography is an option in the purification of a pharmaceutical product. EBA chromatography is possible with unfiltered fermentation medium. However, a possible drawback is the aggregation of the particles in the chromatography column leading to a reduction in the recovery. By measuring this aggregation in-line and adjusting or controlling the washing steps, it may be possible to use EBA chromatography in a purification process. Such an in-line measurement is described in the literature and measures the RTD (Residence Time Distribution) of a tracer in de column. In the turbid medium a selective detector is required. KCl measurement with an ISE (Ion Selective Electrode) is a possible solution (16). This ISE will have to be calibrated and the calibration validated during its in-line use.

The in-line calibration of an EC (Electrochemical) detector is described in an article from Brill and Stover (17). They calibrate the detector in-line by repeatedly injecting different concentrations of a standard solution and calculating a calibration line. They then try to determine how frequent this calibration procedure is required. The slope and the intercept are two correlated parameters that are analyzed by Hotelling T^2 and a PCA (Principal Component Analysis). A third prototype application was build to address this issue. In this application real-time data is first collected and stored in an Oracle database. An external component and the Modbus protocol was chosen to read the data using a COM port. In a control chart this real-time data can be visualized and intervals can be selected.

An interval from an analysis with a new detector can be used to calculate the control limits. These control limits can serve in a Hotelling T^2 control chart to validate the calibration of the detector. Hotelling T^2 statistics is the multivariate

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generalization of Student t-statistics for the evaluation of the significance.

PCA with the slope and the intercept as the two components was used as another option. Principal component analysis can be used to visualize a large amount of information in the plots of the first PC's and to compress data from the original variables to significant components (10). In this simple example the slope and the intercept are compressed to one PC that can be monitored in a control chart.

CONCLUSION

Analytical method validation consists of a number of familiar components. To streamline a specific analytical method validation, a number of base modules in the form of SAS EG add-ins can be used as starting points for the automation of method validation. Together they can form part of a flexible workflow-based, configurable system that implements SOP-based validation practices (4).

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Figure 1.

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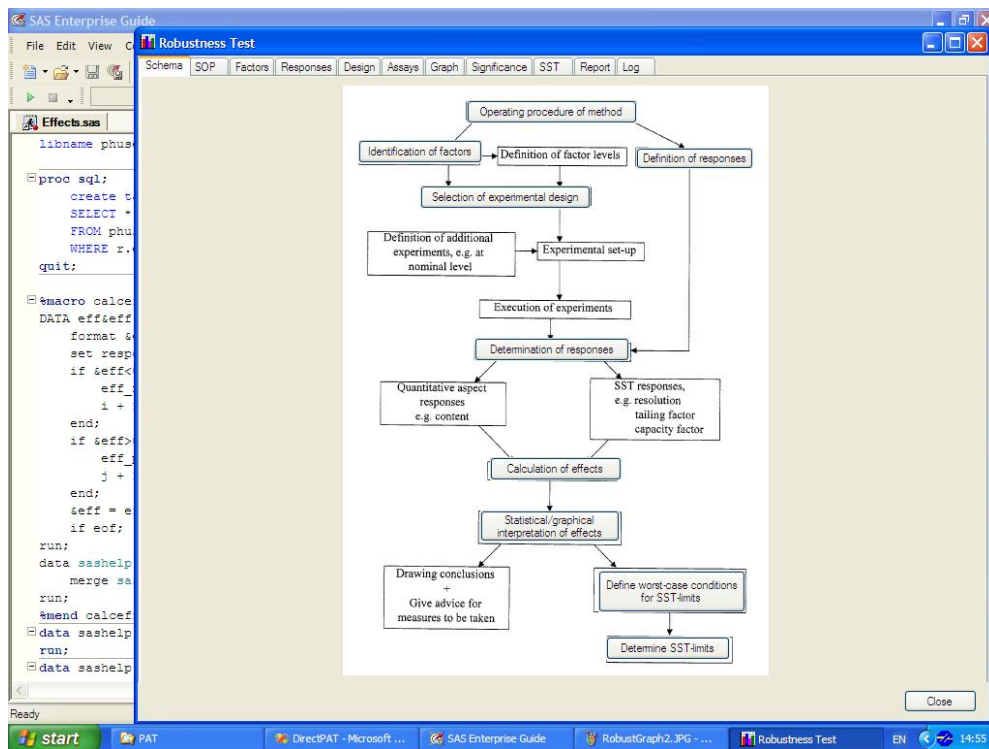


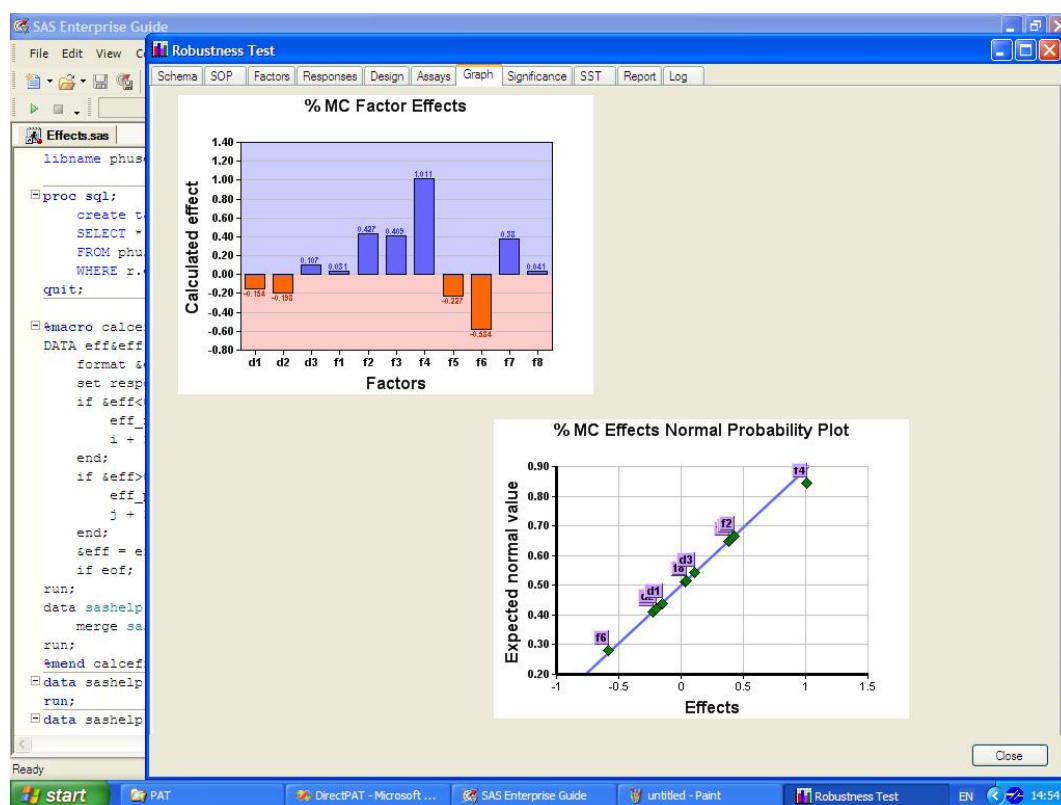
Figure 2.

The table displays experimental results for 12 experiments. The columns represent various factors and responses, including % AC and Rs(AC-RC1).

Exp	(2)	(4)	(D1)	(3)	(5)	(6)	(D2)	(1)	(8)	(7)	(D3)	% AC	Rs(AC-RC1)
1	1	1	1	-1	1	1	-1	1	-1	-1	-1	101.60	5.6910
2	1	1	-1	1	-1	-1	1	1	1	1	-1	101.70	7.4840
3	1	-1	1	1	-1	1	-1	-1	-1	1	1	101.60	5.77
4	1	-1	-1	-1	1	1	1	1	1	1	-1	101.90	5.0250
5	1	-1	1	-1	-1	-1	1	1	1	-1	1	101.80	5.44
6	-1	1	1	1	1	1	1	-1	1	-1	-1	101.10	5.7110
7	-1	1	-1	-1	-1	-1	1	1	-1	1	1	101.10	5.9320
8	-1	-1	-1	-1	1	1	-1	-1	-1	-1	1	101.60	4.9620
9	-1	-1	1	1	1	-1	1	1	-1	1	-1	98.40	5.4270
10	-1	1	1	-1	1	-1	-1	-1	1	1	1	99.70	6.3440
11	1	1	-1	1	1	-1	-1	-1	-1	-1	1	99.70	6.7150
12	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	102.30	5.1860
Responses	Effects											Critical dummies	effects Dong
% AC	0.683	-0.450	-0.683	-0.717	-1.117	0.883	-0.750	-0.017	0.517	-0.617	-0.250		
Rs(AC-RC1)	0.427	1.011	-0.154	0.409	-0.227	-0.584	-0.198	0.031	0.041	0.380	0.107		

Figure 3.

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