A check list for multi-instrument projects

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INTRODUCTION

Goal of this check list

Modern multivariate data analysis such as those developed in chemometrics makes it possible to solve many practical problems that previously were very difficult to address with proper scientific validity.

However, R&D in real-world, complex systems requires that the system is studied from sufficiently many angles at the same time: Whenever possible, one should employ multi-channel instrument, and one should combine information from two or more instruments.

Combining several types of instruments usually means combining the efforts from several people, often from several scientific disciplines. Serious coordination problems of technical or human nature may then arise.

Proper project planning may reduce the probability of such coordination problems. This data analysis check list is a tool for facilitating ”multi-information-type” projects, i.e. R&D projects where data of several types are to be combined.

The present check list reminds the user of a series of links in the planning process. It includes:

- Interdisciplinary goal formulation and choice of analytical methods

- Statistical experimental planning to select samples with necessary and sufficient precision of information relevance to the goals
- Organisational planning of resource allocation and timing to ensure productivity

Efficient DO-It-Yourself data analysis:

The planning check list stresses that initial hypotheses and a priori expectations of the outcome should somehow be made visible and explicit. This can reduce the probability of misunderstanding between experts from different fields involved in the project. It can also serve to reveal unclear points in the original idea behind the project before it is too late.

It is a sad fact that many laboratories grossly underestimate the uncertainty in their results; this is evident in most multi-laboratory comparisons (round-robin tests): Systematic effects such as errors in reference standards, personal bias and long term instrument drift are often ignored.

A second sad fact is that uncertainties are difficult to estimate statistically, even when all uncertainty sources have been correctly included in the experiments. Estimation of uncertainty standard deviations require a high number of observations to become reasonably precise. To give statistical confidence limits is even more demanding, because then the statistical distribution between the measured values must also be evaluated[1].

A third sad fact is that many chemists and other non-statisticians find mathematical statistics difficult. Incorrect use of statistics is therefore a problem; feeling of alienation towards one´s own results is another problem.

Therefore, data analysis should not be made more mathematical or statistical than what is necessary. Modern experimental planning and multivariate chemometrics/qualimetrics help scientists draw valid extraction and interpretation of large amounts of experimental data without difficult statistical theory. We have in the preceding paper[2] discussed the advantages of applying multivariate chemometric methods, and readers not familiar with multivariate methods may refer to this paper for further discussion of the subject. More details, within the same notation and terminology framework, is given in Martens & Naes (1989)[3], Esbensen, Schoenkopf and Guyot (1998)[4]

The check list is intended to increase the efficiency of analytical laboratories in general, and especially laboratories involved in multidisciplinary research projects.

THE CHECK LIST USED IN A CASE STUDY

We have ourselves found the ensuing check list helpful in a collaborative project with four

different research institutions and two industrial companies. Among the four research institutions, three were university institutions and one was a governmental research institution. Only one of the industrial partners were directly involved in the analytical part of the project. This company has a large research and development department, who participated in the project. In the project a number of storage experiments were carried out on mayonnaise containing fish oil and various antioxidants and with different physical structure. Different measurements on the same samples were made by the participants. Nine completely different analytical methods were applied namely sensory analysis, electron spin resonance detection of free radicals, HPLC detection of lipid hydroperoxides, GC-MS detection of volatile compounds, droplet size determinations by laser diffraction and by confocal laser scanning microscopy and rheological measurements (three different methods) and more than 200 variables were measured. Multivariate data analysis was used to interpret the data. The nature of the analytical and data analysis problems encountered by the various laboratories will briefly be described as well as the measures taken to solve the problem. In all cases the improvements of methods and procedures were worked into the ensuing versions of our checklist.

Sensory evaluation of mayonnaise: Larger uncertainty standard deviations than specified in the original project checklist (20 %) were observed for most of the sensory attributes employed. This had also been a problem in the past, but due to the checklist the problem was elucidated and more focus was therefore put on the problem in the present project. The large standard deviations indicated that the assessors used the sensory scale differently. The absence of one assessor in one of the sensory sessions may therefore have a large impact on the result of that particular sensory session. Therefore, differences in the sensory score levels were removed [5]. Furthermore, calculations of the signal/noise ratio of each assessor were calculated to evaluate the performance of the assessors.

Electron spin resonance: One of the objectives of the data analysis check list is to write down hypotheses for the project work. In our case this made one of the participants write down the reactions that may take place when using electron spin resonance- spin trapping for the detection of free radicals in emulsions supplemented with different antioxidants. Thereby, he realized that possible side effects could make the measurements useless. Thus, the theoretical assessment revealed that for some antioxidants the ESR-method may not be applicable because the signal may be quenched by the antioxidant. Consequently, this was always examined before testing new antioxidants in large experiments.

HPLC detection of lipid hydroperoxides: During the testing process of the reproducibility of the HPLC method, as specified in our original check list, it was observed that the intra-assay variation increased over time, indicating that the time from the sample was placed in the autosampler to the time when the sample was injected had an effect on the measured concentration of lipid hydroperoxides. Precautions were therefore taken to avoid this problem. The problem would probably also have been discovered without the project checklist, but the check list helped focusing on the problem.

Sampling procedure: Mayonnaise samples were stored in glass jars. During the oxidation

process, the oxidation products may not distribute uniformly throughout the sample due to the high viscosity of mayonnaise. One of the project participants was not aware of this problem and therefore mayonnaises were not mixed before samples were taken for electron spin resonance analysis. Large standard deviations were observed in some samples. The fact that the sample preparation is described in the project checklist helped the other project participants to uncover the problem and the solution to the problem, namely a thorough mixing of the mayonnaise before sampling, was worked into the checklist. The focus on the sample preparation in the project checklist was also used in connection with interpretation of unexpected results obtained by he HPLC-method. Thus, it was observed that the peroxide value (PV) in some instances did not develop according to the usual pattern; namely increasing values followed by decreasing values. Rather, PV in some instances decreased, increased and then finally increased. Samples for HPLC measurements were prepared by freezing the mayonnaise, whereby it separated into an oil phase and an aqueous phase. Initially, as much as the oil phase as possible was poured into a centrifuge glass and then centrifuged. However, this sampling procedure may not be reproducible, because the amount of aqueous phase (which also contains lipids) used for centrifugation may vary and this may perhaps affect the results. Therefore, in order to obtain a representative sample the sampling procedure was changed so that the thawed mayonnaise was vigorously shaken before being poured into the centrifuge glass.

Dynamic headspace GC-MS to determine secondary oxidation products: Initially this analysis was made in triplicate, but in some cases the reproducibility was not satisfactory (relative standard deviations above 20 %,- higher that originally specified in our check list). The headspace sampling method was improved, which reduced the relative standard deviation. Furthermore, the number of replicates were increased to 4. Based on the relative standard deviations the three best measurements were selected for further data analysis.

Determination of droplet size by laser diffraction measurements: Measurements were made after 1 and 4 weeks of storage. As part of the check list's required quality control of the running experiment, extremely large increases in the droplet size were observed between 1 and 4 weeks of storage in some samples in the first experiment. Similar increases were not observed when the same samples were viewed in a microscope. Once more the focus was put on the sample preparation step. In the first experiment, mayonnaises were diluted with distilled water before measurement, which apparently did not separate agglomerates of oil droplets. Therefore, the dilution step was improved by employing SDS buffer instead of water[5] and this solved the problem.

Compatibility of software: Initially the participants in the project did not use the same software for wordprocessing, spreadsheet etc and this gave rise to problems when exchanging textfiles and spreadsheets. Therefore all participants agreed on one type of software. The issue of compatibility of software was not included in the original version of the model checklist, but due to our experiences this issue was added to the chekclist.

Another purpose of the data analysis check list is to write down *a priori* expectations of the outcome of the experiment, e.g. the expected correlation between variables.

The number of variables measured was too high to make the visual inspection of the raw data or assessment of individual correlation coefficients fruitful. We used instead Partial Least Squares Regression (PLSR) to analyze the data. We noted *a priori* that we expected to find a positive correlation between the viscosity and the sensory variables describing the texture of the mayonnaise. However, no correlation between the viscosity and the texture variables was visible in the PLSR mapping of one of the data sets. This made us go back and look at the raw data again and we found that the data for one of the samples were wrong by a factor 10. After having analyzed the data again using the correct values we observed a positive correlation between the sensory and viscosity variables as expected. The example illustrates how the *a priori* expectations, made explicit in the check list, in combination with multivariate data analysis, can be used to discover erroneous raw data. The present error could also have been discovered by other methods, but probably not as quickly

In our project work multivariate data analysis was a very useful tool. In fact, it would have been very difficult if not impossible to analyze the relations between the many variables without this data analytical tool. In addition to being able to analyze the relations between the many variables, the multivariate data analysis also made it possible to analyze the effect of the antioxidant additions and changes in processing conditions (i.e. experimental design) on each of the many variables. Based on this understanding of relations between the variables and the effect of the experimental design we were able to propose explanations for the mechanisms behind the observed variation in the data. Subsequently, we carried out new confirmative experiments to test if our hypotheses were correct, and the results were satisfactory[6].

The problems used to illustrate the advantages of applying our checklist in a interdisciplinary project could also have been solved without the checklist. However, the checklist helped the whole project group to focus on the problem. Furthermore, the solutions to the problem were worked into the ensuing versions of the checklist, and this ensured that the improved methods were communicated to all partners in the project and not hidden in a desk drawer.

References:

1. ”Guide to the expression of uncertainty in measurement (GUM)”. (1995) ISO Geneva, ISBN 92-67-10188-9,
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3. Martens H. and Naes, T.(1989) Multivariate Calibration. J.Wiley & Sons, Ltd.
4. Esbensen, K. Schoenkopf, S. and Guyot, D. (1998) Multivariate Analysis in Practice. CAMO ASA, Oslo, Norway.
5. Jacobsen, C. Hartvigsen, K. Lund, P, Meyer, A.S., Adler-Nissen, J. Holstborg, J. and Hølmer, G. (1999) Oxidation in fish oil enriched mayonnaise: 1. Assessment of propyl gallate as antioxidant by discriminant partial least squares regression analysis, Z. Lebensm. Unters. Forsch. (In press).
6. Jacobsen, C. (1999). Oxidation mechanisms in fish oil enriched emulsions. PhD thesis, Technical University of Denmark, Institute of Biotechnology.

Check list, multi-instrument project planning

1. Project description

1.1 Project name:

1.2 Overall goals for the project:

1.3 What is the relation between the goals of the project and the problems that the project is intended to solve?

1.4 Hypotheses/expected results of the present experiment:
(Try to be as specific as possible. But in purely explorative projects, it may be enough just
to state the main ideas.)

1.4 Describe your level of ambition with your mathematical modeling at the present stage of the project:

- Explorative data analysis (multivariate, interactive soft modeling),or
- Confirmative, statistical hypothesis-test (statistical hard modeling) or
- Causal description (deterministic hard modeling e.g. based on physical/chemical mechanisms) ?

1.5 Relevant references & previous reports:

2. Project planning

2.1. Preparing the different measurement types:

Measurement type 1:
a. Name of measurement:
b. Person responsible:
c. Resource persons:
d. Purpose
e. Principle (refer to theoretical principle):
f. Procedure (refer to description of how principle is used):
g. Known interferences:
h. Analytical capacity including data analysis of raw data (samples/day):
i. Date for Test Version, this measurement type:
j. Date for Final Version, this measurement type:
k. Measurement method (refer to detailed method description):
k.1. Sample preparation:
k.2. Channels used (variables to be measured? In which unit measurement?):
k.3. Instrument standardization (how often is the instrument to be standardized or

calibrated, and how?):

l. If the instrument changes its performance in the middle of your experiment, due to e.g. maintenance, would you then be able to restandardize it to be compatible with previous measurements (yes/no)?

m. Describe the plans for statistical process control of the measurements and for checking of instrument performance:

n. Main sources of uncertainty in this measurement type (e.g. time from preparation to measurement, leakage of gas in systems where no leakage is allowed):

n.1 _____
n.2 _____
n.3 _____
n.4 _____

o. Characterization of the resulting expected uncertainty types:

o.1 Repeatability. What is the expected standard deviation between successive measurements of the same measurand carried out under the same conditions of measurement (same measurement procedure, same observer, same instrument, same location, short period of time)?

o.2. Reproducibility. What is the expected standard deviation between the results of measurements of the same measurand carried out under changed conditions of measurement?
Which conditions were kept constant, and which were changes?

o.3. Accuracy & tracability. What is the closeness of the agreement between the result of the measurements and a value of the measurement regarded as "true" (qualitative statement, relative to the goals and known needs of the project)?

Measurement type 2:
(Copy check list from measurement type 1)

2.2 Choice of sample types

a. Person responsible:
b. Resource persons:
c. Type of objects to be sampled (products from real industrial process, products from pilot plant, products produced in laboratory according to recipe, or simplified laboratory model system):
d. How relevant is the model system in regard to the goals of the project?:
e. Which known aspects of properties of the real world objects cannot be studied in this model system?:
f. Date for Test Version, Plan for choice of sample types:
g. Date for Final Version, Plan for choice of sample types:

2.3 Experimental design for selecting the individual samples and performing the measurements

a. Person responsible:
b. Resource persons:

c. Preliminary Pilot experiments:
d. Which main phenomena can influence measurements (f.ex. differences in processing conditions, sensory panel variance)?:
e. Which of these phenomena are controlled to be kept constant ?
f. Which of these phenomena cannot be controlled?
g. Which of these phenomena are to be varied in a controlled way as design factors?
h. For each of the design factors:
h.1) What is its maximum range of variation that is relevant?
h.2) What is its minimum variation deemed necessary to cause a reliably detected effect by the analytical instruments?
i. Requirement for random sampling, in order to pick up unexpected and uncontrolled phenomena:
j. Final experimental design:
(Factorial design, response surface design, mixture design, or pragmatic combination hereof.)
k. Are the sampling and the measurement order sufficiently randomized to separate time drift from the experimental factors?
l. How can this experimental design plan be extended, if this is found necessary?
m. Date for preliminary experimental design:
n. Date for final experimental design:

2.4 Data conversion: Bringing all types of data to one common file format
a. Person responsible:
b. Common file format:
c. Date for having checked that all data types can be converted to this file format:

3. Experimental work

3.1 Acquiring samples for measurement:
a. Person responsible:
b. Resource persons:
c. Schedule for sample acquisition:
d. Date for having acquired all samples:

3.2 Measurements according to experimental plan:
a. Person responsible:
b. Resource persons:
c. Schedule for measurements:
d. Date for having finished all measurements:

4. Data analysis

4.1 Quality control as the data emerge from each instrument type

Measurement type 1:
a. Person responsible:
b. Resource persons:
c. Data analytic method:
d. Software program:
e. Memory requirements for making calculations on raw data:
f. Disk space requirements for storing raw data and results:
g. Computing capacity requirements for making calculations:
h. Potential problems (f.ex. breakdown of important instrument) and fall-backs:
i. Criteria for alarm (control chart is recommended):
j. Final date for having the routine data analysis method ready:

Measurement type 2:
(Copy checklist for measurement 1)

4.2 Interdisciplinary data analysis
a. Person responsible:
b. Resource persons:
c. Data analytic methods:
d. Modeling method e.g.:
- For explorative analysis: f.ex. bilinear modeling PCA or PLSR:
- For confirmative analysis f.ex. ANOVA:
- For causal analysis f.ex. kinetic or equilibrium models:

e. Strategy for combining different types of input data:
e.1 Is there a need to weigh (scale) the different input variables differently, because they have different uncertainty levels? How?
e.2 Is there a need to weigh (scale) the variables to see all of them properly in the graphical display of the results? How?
f. Strategy for handling outliers:
g. Human interpretation methods (f.ex. graphic interpretation for PCA analysis)
h. Validation principle (in addition to visual interpretation)
- Cross validation/jackkniffing
- Statistical significance test (t-test, F-test)

(If many different variables are to be tested independently for significance, how many of them are expected to appear as "significant" just by chance?)

i. Which validation levels are to be attempted in the case of multivariate data analysis?
i.1 Repeatability of the measurements

(e.g. cross validation between replicates, or fixed model in ANOVA)

i.2 Predictive ability: Ability to predict new, unknown samples of the same general kind
(e.g. cross validation between independent products or samples)

i.3 Reproducability of the results
(e.g. full cross validation between independent results from different people, different instruments, different points in time or different labs, or mixed model in ANOVA)

j. Internal and External validation of data analytic interpretation:

- Internal validation: Check that the measurements reflect that which was intended
- External validation: Can the results be supported by independent evidence?
(e.g. such as both good statistical predictive ability and cognitively plausible results)

k. Software program:
l. Memory requirements for performing full data analysis on your specific data :
m. Disk space requirements for performing full data analysis on your specific data :
n. Computing capacity requirements for performing full data analysis on your specific data:
o. Potential problems known:
p. Criteria for alarm (outlier analysis, control chart etc):
q. Criteria for success:
r. Schedule for learning to use the final data analysis method:
s. Schedule for testing the final data analysis method:
t. Final date for having the final data analysis method ready to receive real data:

5. Conclusions/Reports

5.1. Which text editor and graphic presentation system will be used and are they compatible with each other?:

5.2 Type 1 report
a. Persons responsible:
b. Resource persons:
c. Target groups:.
d. Deadline:
e. Presentation forum:
f. Authors:
g. Important points:
h. Presentation level:
i. Presentation method:

Type 2 report
Copy checklist for Type 1 report

5.3 Patents from project work:

a. Persons responsible:
b. Resource persons:
c. Deadline:
d. Type of patent:
e. Inventors:
f. Final date for preliminary filing:
g. Important points: