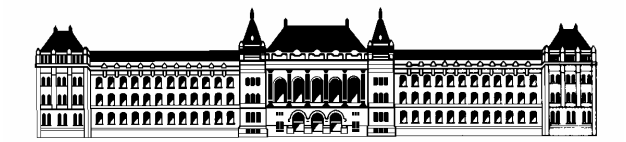

BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS

FACULTY OF CHEMICAL AND BIOENGINEERING

Theses of the Ph.D. dissertation entitled

Statistical evaluation of pharmaceutical stability data

submitted by

Kinga Lakné Komka
M.Sc. in bioengineering

under the supervision of

Dr. Sándor Kemény
professor

Department of Chemical and Environmental Process Engineering

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1. Introduction

Every drug product on the market has to apply for registration. One of the most important parts of the several hundred page documentation of the registration process is the detailed description and the statistical evaluation of the stability study of the pharmaceutical product. It is very important for the pharmaceutical companies to establish accurate, reliable and the longest possible shelf-life for their drug products. The adequate and properly applied statistical methods help to reach these requirements.

The current method of the statistical evaluation of the stability data has been already studied in the course of my diploma work[1]. The experiences gained then, contradictions in the literature and the need of the pharmaceutical companies were my motives to immerse in the theme and to elaborate novel, more accurate and reliable statistical methods for the shelf-life estimation.

2. Literature review and the aim of the work

The purpose of the stability study is to provide evidence on the variation of quality of a drug product with time under the influence of a variety of environmental factors such as temperature, humidity and light, and to establish the shelf life for the drug product and for the recommended storage conditions.

A complete stability design includes two types of testing: the long-term stability study, which is conducted under intended storage condition and the accelerated stability study under stressed conditions (at higher temperature and relative humidity). My PhD work focuses only on the long-term stability testing.

[1] Kinga Komka: The statistical evaluation of pharmaceutical procedures, *Diploma work*, BME Department of Chemical Engineering, 2000 (in Hungarian)

For the long-term stability study, a random sample of tablets is taken from several batches. The tablets are stored under controlled temperature and humidity conditions and periodically (every 3 months over the first year, every 6 months over the second year, and annually thereafter through the proposed shelf-life) examined their physical, chemical, pharmaceutical and microbiological attributes. My PhD work concentrates mainly on the active ingredient content analyses. To take into consideration the unavoidable fluctuation of the manufacturing process, at least three batches of the drug product should be examined.

Two ICH guidelines[2,3] contain the current regulations for the design and the statistical evaluation of the stability study valid for all the three regions of Europe, Japan and the United States.

The expiration dating period (shelf-life) is defined as the time interval until the drug product is expected to remain within the specifications established for its strength, quality and purity. According to the ICH Guideline for an attribute known to decrease with time (for example the active ingredient content), the shelf-life is calculated as the time point at which the lower one-sided 95% confidence limit for the mean degradation curve intersects the acceptance criterion. In terms of an attribute known to increase with time (for example the concentration of an impurity) similar definition can be given, but for the upper limit. Thus the estimation of the shelf-life is based on the adequate degradation line fitted on the stability data and the on the appropriate statistical interval.

In my PhD work I deal only with linear degradation relationship. The statistical model of the actually valid ICH Guideline is a general linear model with one categorical factor (the batch factor) and one continuous variable (the storage time).

[2] ICH Harmonised Tripartite Guideline Q1A(R2): Stability Testing of New Drug Substances and Products (Second Revision), 2003

[3] ICH Harmonised Tripartite Guideline Q1E: Evaluation for Stability Data, 2003

The fluctuation of the measured stability data is composed of four different sources (batch-to-batch and the within batch variability, the reproducibility and the repeatability of the analytical method). In the course of the statistical evaluation the incomplete knowledge of the reality is considered by a statistical interval for the fitted degradation line. As statistical interval the ICH Guideline recommends the confidence-bound.

Many points of the current regulation of the ICH Guideline are subjects of debate in the literature. From among these points two important questions are mentioned, which are also essential subject of my PhD work:

- the number, the character (fixed or random) and the structure (crossed or nested) of the factors in the statistical model of the stability study
- the adequacy of the confidence bound approach or using another statistical interval instead

Summarizing these facts the aim of my PhD research work is the critique and improvement of the current statistical model of the stability study for the sake of the reliable shelf-life estimation.

3. Computation methods

The statistical evaluation of the pharmaceutical stability data applies the following topics from the mathematical statistics: regression analysis, analysis of variance (ANOVA), general linear model (the combination of the two previous topics) and the computation of the several statistical intervals (confidence-, prediction and tolerance interval).

The statistical calculations - if not specified in a different way - were performed with the software package STATISTICA (version 6.1)[4], which is applied generally in our research group. However, there were some statistical

[4] StatSoft, Inc. (2003). STATISTICA (data analysis software system), version 6.1. www.statsoft.com.

problems in the course of my research work, for which the software package STATISTICA was not sufficient. The new definition of the factor of the date of analysis (chapter 4.1.) resulted a mixed model, for which a Visual Basic program (written within the frame of STATISTICA) had to be also used and the results were compared with the results of other statistical software (SAS, SPSS). The simulation study for the stability models applying tolerance intervals (chapter 4.2.) was performed with a program written in MATHEMATHICA software[5].

4. New results

My research work is divided into three parts according to the subject of the statistical evaluation of the pharmaceutical stability data. The aim of the first two topics is to improve and reformulate the current statistical model. The third theme raises a new question: possibility of the estimation of the variation of the stability data in advance (before the start of the stability study).

4.1. New definition of the factor of the date of analysis in the statistical model of the stability study

The statistical model of the ICH Guideline considers only two effects: the difference between the batches and the degradation during the storage. The other fluctuation sources of the stability data are described with one random error term. Thus the model leaves the interval-to-interval variability of an analytical method out of consideration, however generally the reproducibility is the most significant source of the fluctuation of the pharmaceutical stability data.

[5] Wolfram Mathematica. version 6.0.0 (2007)

From among the literature proposals for the consideration of the interval-to-interval reproducibility in the statistical model the most important work is the Norwood-model[6], which extends the statistical model with a new factor, the date of analysis (δ_{ij}):

$$y_{ijk} = \mu_i + \beta x_{ij} + \delta_{ij} + \varepsilon_{ijk},$$

where y_{ijk} is the k^{th} replicate assay measurement of the i^{th} batch at the j^{th} time point

μ_i is the effect of i^{th} batch, i=1,…,r

β is the degradation rate

x_{ij} is the storage time, j=1,…,n_i

δ_{ij} is the error component associated with the batch-age combination (namely the reproducibility error)

ε_{ijk} is the error component within a batch-age combination (namely the repeatability error)

In the Norwood-model each batch-time combination (*ij*) is a different level of the date factor. This means that the model considers different levels of the reproducibility error for different batches at a given time point. However, at a certain date point the circumstances of the analysis (e.g. analyst, instruments and reagents), thus the reproducibility error is the same for all batches. Summarizing these facts the reproducibility error has to be an independent (not a nested) factor from the batch.

My proposed model is based on the Norwood-model, but its aim is the adequate consideration of the reproducibility error. This comprises new definition of the factor of the date of analysis.

In my model the date factor has only one subscript (*t*), different from the subscripts of the other terms:

$$y_{ijk} = \mu_i + \beta x_{ij} + \delta_t + \varepsilon_{ijk},$$

[6] Norwood, T.E.: Statistical analysis of pharmaceutical stability data, *Drug Development and Industrial Pharmacy*, 12(4), 553-560 (1986)

where the meaning of the variables - except the δ_t factor - agrees with the list at the Norwood-model. The new subscript (*t*) of the factor of the date of analysis refers the specific time point (the date) of the analytical measurement, so the δ_t factor means the reproducibility error at a given time point, which is independent from the batch.

It's very important, that applying this new definition of the factor of the date of analysis two time scales appear in the model, which is a quite new concept. The date scale (*t*) is fixed to the calendar time and joins to the random effect attributed to the interval-to-interval variability. The storage time (*j*) is defined as the time interval which has passed from the start of the stability study and belongs to the degradation rate. The two time scales are not the same when the batches are put to storage at the different time point. However this is not a general practice in the pharmaceutical industry, but its results can be significant: it can extend the estimated shelf-life.

Incorporating the random date effect to the ICH Guideline model, which contains only fixed effects, a mixed model is obtained, solution of which is more difficult. For the statistical evaluation of the new model the available STATISTICA software was not enough, its Visual Basic program has to be also used.

The results of the research are summarized in ***Thesis 1***.

4.2. The estimation of the shelf-life with an extended tolerance interval model

For the shelf-life estimation of pharmaceutical products the ICH Guideline recommends the confidence bound as the calculated statistical interval reflecting the fluctuation of the stability data. The confidence bound approach assumes a

homogenous population of tablets and a true degradation line valid for all tablets, which may only be estimated due to the analytical measurement error.

The model: $y_{ijk} = \mu_i + \beta x_{ij} + \varepsilon_{ijk}$,

where the meaning of y_{ijk}, μ_i, β és x_{ij} variables/parameters are the same with the list in Section 4.1., ε_{ijk} random error stands for the measurement error.

For the shelf-life estimation the tolerance interval concept is proposed instead of the less proper confidence bound approach. The tolerance interval takes the manufacturing difference between the tablets into consideration and contains at least a specified proportion of the tablet-population (e.g. 99%) with a high degree of confidence (e.g. 95%). The model itself is the same, but for effect of the ε random error a tolerance interval is calculated instead of a confidence interval.

Former literature proposals[7,8] for applying tolerance interval for the shelf-life estimation use incorrect formulae for calculations. Parallel to my initial research, Kiermeier et al.[9] also propose the tolerance interval concept using for the stability studies, in a proper formulation. The tolerance interval model of Kiermeier, however, contains only one fluctuation source, namely the tablet-to-tablet (within batch) variability. The measurement error is supposed small and neglected as compared to the tablet-to-tablet variability. In contrast with Kiermeier's concept, my tolerance interval model considers two influence sources within a single batch: the tablet-to-tablet variability and analytical measurement error. The structure of my model makes the incorporation of other factors (e.g. the batch to batch difference) easy.

[7] Chow, S.C.; Shao, J.: Estimating drug shelf-life with random batches, *Biometrics*, 47, 1071-1079 (1991)

[8] Shao, J.; Chow, S.C: Statistical inference in stability analysis, *Biometrics*, 50, 753-763 (1994)

[9] Kiermeier, A.; Jarett, R.G., Verbyla, A.P.: A new approach to estimating shelf-life, *Pharmaceutical Statistics*, 3, 3-11 (2004)

The new model is similar to that mentioned at the beginning of this section, but the tablet-to-tablet variability (δ_{ik})also appears in it as a random factor and if several batches are investigated, the μ_i batch factor may also be random:

$$y_{ijk} = \mu_i + \beta x_{ij} + \delta_{ik} + \varepsilon_{ijkl} .$$

It should be mentioned that tolerance interval models containing two variation sources (tablet-to-tablet variability and analytical measurement error) require that several samples be taken at the time points (*k* subscript in the model) and the analyses be repeated (*l* subscript in the model), otherwise the numerical separation of the two influence sources is not possible.

Another, in the literature is not yet examined, question is whether the tolerance interval should be constructed for the true or the measured assay content. My opinion is that the tolerance interval needs to focus on the true assay content, because the patient is interested in the true assay content, not in the measurement result subject to error due to chemical analysis.

Based on the considerations above the following hierarchically built tolerance interval models are constructed:

- the simplest stability model, containing one variation source only ("A_1" model), here the within batch variability (δ) is not considered;
- the stability model for the *measured* assay content, considering two variation sources ("A_2" model), here both measurement error and within batch variability (δ) are considered;
- the stability model for the *true* assay content, considering two variation sources ("A_2^*" model), here the within batch variability (δ) is considered for the tolerance range, but the ε analytical error not;
- the stability model considering also the batch variability, here the μ_i random batch factor also appears.

With the models above a simulation study (applying MATHEMATICA software) was performed. The aim of the simulation was to study the statistical behaviour of the models with special regard to the coverage probability (e.g. that the interval, in which the assay content of the 99% of the tablets with 95% confidence is found, contains the results the same probability indeed) and the width of the tolerance interval at the specific models and parameter-combinations. From among the parameters influencing the width of the tolerance interval the following were examined:

- the number of the samples and the number of the repeated analyses from a sample at each time point
- the assumed variances of the tablet-to-tablet variability and the analytical measurement error, respectively

In principle the width of tolerance intervals (and thus the shelf-life values) for model variants might be compared analytically, but this is tedious because of the approximations (e.g. Satterthwaite-approximation) applied in the models. For this reason the nowadays generally applied and accepted simulation approach was chosen.

The novelty of the proposed stability models based on the tolerance interval concept is stated in ***Theses 2 and 3***, and the results of the simulation study are summarized in ***Thesis 4***.

4.3. Estimation of the uncertainty of the stability data in advance

It is an essential interest for the pharmaceutical companies that the estimated shelf-life of their drug products should be the longest possible. The highly variable stability data however reduce the estimated shelf life.

If the magnitude of the fluctuation sources of the stability data is known in advance, we have information before the start of the stability study on the

expected uncertainty of the future stability data. Based on this estimate value one may consider whether the fluctuation of the present manufacturing process and analytical method is suitable for the stability study. This would be very useful for the pharmaceutical companies, because it will be apparent in advance, that the drug product probably fails in the course of stability study and the manufacturing process and/or the analytical method has to be improved.

To reach this goal a calculation method is elaborated, by which all of the uncertainty components can be estimated before the start of the stability study and their effect on the shelf life estimation is considered in advance. The advantage of my method is that no extra chemical analysis is required to the calculation, because the results of the analyses normally performed during the analytical method development procedure (the content uniformity test and the validation of the analytical method) are used for the estimation of the elementary variance components. The statistical method for the computation is the one- and two-way ANOVA and the variance component analysis.

Using the estimates of the variance components concerning each fluctuation source the expected width of the confidence-interval is estimable, this value strongly influences the estimated shelf-life.

The essence of this new calculation method is summarized in ***Thesis 5***.

5. Theses

Thesis 1

In the statistical model of the stability study of the pharmaceutical products Norwood method was improved considering the error caused by the date of analysis. In the context of the ICH Guideline, namely applying the confidence bound approach a crossed design is applied instead of Norwood's nested model.

Simultaneously the number of level of the factor of the date of analysis has been also changed: the levels are fixed to the sampling times.

The novel model - in contrast with the models in the literature - contains two time scales, which make the separate consideration of the storage time and the date of analysis possible [2].

Thesis 2

At the shelf-life estimation of the pharmaceutical products the tolerance interval concept is proposed for the consideration of the fluctuation of the stability data instead of the confidence bound approach recommended by the ICH Guideline.

Instead of the literature models containing only one variation source, a tolerance interval model included several factors is built, which consider the two influence sources within the batch, the tablet-to-tablet variability and the analytical measurement error [1].

Thesis 3

First in the literature I proposed, that the tolerance-interval model for the stability study of the pharmaceutical products concerns the true (and not the measured) assay content.

The width of the tolerance interval models containing two influence sources depends on the choice between the measured or the true assay content for investigation. The tolerance interval for the true assay content is narrower than that for the measured assay content [1].

Thesis 4

Studying the statistical behaviour of the stability models based on the tolerance interval concept with simulation, I stated [1], that

A. If there are two distinct influence sources present, the models containing two influence sources describe well the simulated cases (the value of the coverage probability was close to the nominal 0.95), while the model containing one (overall) variation source is not suitable for the adequate description of the cases simulated assuming two influences sources (the value of the coverage probability was much below 0.95). Thus it can be stated that since in real stability studies always more fluctuation sources are present, the former literature models containing only one variation source are not adequate for the reliable shelf-life estimation.

B. Investigating the effect of the different combinations of the variance component values, I found that the higher the assumed overall variance, the wider the tolerance interval is and in case of fixed total variance the tolerance interval becomes narrower if the tablet-to-tablet variability is smaller than the analytical measurement error.

C. Applying different number of samples and repeated analyses I found that increasing the number of the samples from a batch at a given time point the tolerance interval becomes significantly narrower, but with the increasing the number of the repeated analyses it does not. This means that at the planning of a stability study it will be profitable to assign the number of available repetitions in the way that at each time point the highest possible number of samples are analysed with fewer replicates in contrast to the common pharmaceutical practice, where the number of the samples and that of the repeated analyses are usually the same.

Thesis 5

I elaborated a calculation method by which the fluctuation of the stability data is forecasted. To the computation the results of the validation performed at the development of the analytical method and the heterogeneity-information coming from the manufacturing practice (or the Content Uniformity test) were used.
With my concept it is possible to decide before the start of the stability study whether the fluctuation of the present manufacturing process and analytical method is suitable for the stability study [3].

6. Applicability of the results

The generally accepted shelf-life estimation procedure regulated by the current ICH Guideline is not flawed, because it does not answer the relevant question. The procedure refers to the change of the expected value of the assay content of the homogenous population of tablets with time, calculating the time point at which it reaches the specification limit. The uncertainty is taken into account by the confidence bound approach. The relevant question is the time point when the assay content of the bulk (most of individual tablets) is within an acceptable limit. The stability models based on the tolerance interval concept answer this question in contrast to the confidence bound approach of the Guideline. This means that the concept of the ICH Guideline must be changed. I found that the application of the proposed tolerance interval model inevitably means the change of the specification limits as well: the same shelf-life (thus the same quality) for a product is obtained by the tolerance interval approach only if the specification limit requirements are milder.

For cases when the start of stability study for the different batches is shifted in time the novel statistical model using two time scales offers the proper way of statistical treatment.

The calculation method elaborated for the estimation of the uncertainty of stability data in advance answers a new question essential for economical consideration. Since it becomes apparent at the early stage of the drug development process if the drug product will probably fail in the course of stability study, one may decide on improving the manufacturing process and/or the analytical method to avoid this built-in failure.

7. Publications

Publications of the theses

Papers in international journals

1. **K. Komka**, S. Kemény, B. Bánfai: Novel tolerance interval model for the estimation of the shelf-life of pharmaceutical products, *Journal of Chemometrics*, 24, 131-139 (2010). **(IF: 1.415)**

2. **K. Komka**, S. Kemény: A modified error model for the assessment of stability of pharmaceutical products, *Chemometrics and Intelligent Laboratory Systems*, 72, 161-165 (2004). **(IF: 1.940)**

3. **K. Komka**, S. Kemény: Effect of the uncertainty of the stability data on the shelf life estimation of pharmaceutical products, *Periodica Polytechnica Ser. Chemical Engineering*, 48(1), 41-52 (2004).

Presentations and posters on international conferences

1. **K. Komka**, S. Kemény, B. Bánfai: Using tolerance intervals for the estimation of the shelf-life of pharmaceutical products, *Conferentia Chemometrica 2009*, Siófok, 2009. szept. 27-30. (poster)

2. **K. Komka**, S. Kemény: Effect of the uncertainty of the stability data on the shelf life estimation of pharmaceutical products, *25th Annual Conference of the International Society for Clinical Biostatistics*, Leiden, Hollandia, 2004. aug. 15-19. (oral lecture)

3. **K. Komka**, S. Kemény: Considering the date of analysis in the statistical evaluation of pharmaceutical stability data, *1st MEDINF International Conference on Medical Informatics & Engineering*, (*Craiova Medicala Journal*, 5(3), 310-313, 2003, ISSN 1454-6876), Craiova, Románia, 2003. okt. 9-11. (oral lecture)

4. **K. Komka**, S. Kemény: Assessment of stability of pharmaceutical products, *Advances in Chromatography and Electrophoresis – Conferentia Chemometrica 2003*, Budapest, 2003. okt. 27-29. (poster)

Presentations on Hungarian conferences or scientific forums (in Hungarian)

1. **Komka K.**: Gyógyszerkészítmények stabilitásvizsgálatának elemzése többfaktoros, tolerancia-intervallumon alapuló modellel, *Klinikai Biostatisztikai Társaság ülése - A 2009. év fiatal biostatisztikusa pályázat keretében*, Budapest, 2010. márc. 19.

2. **Komka K**.: A stabilitásvizsgálat statisztikai modelljének továbbfejlesztése, *BME Vegyészmérnöki és Biomérnöki Karának Doktoráns Konferenciája*, Budapest, 2004. nov. 24.

3. **Komka K.**, Kemény S.: Gyógyszerkészítmények stabilitásvizsgálatának statisztikai kérdései, *MTA Automatikus Elemzés Munkabizottság ülése a Symposium on Computer Applications and Chemometrics in Analytical Chemistry (SCAC2004) konferencia keretében*, Balatonfüred, 2004. szept. 3.

4. **Komka K.**, Kemény S.: Gyógyszerkészítmények stabilitásvizsgálatának statisztikai értékelése, *Műszaki Kémiai Napok'04*, Veszprém, 2004. ápr. 20-22.

5. **Komka K**.: Az analízis időpontjának figyelembevétele gyógyszerkészítmények stabilitásvizsgálatának statisztikai értékelésénél, *Klinikai Biostatisztikai Társaság ülése*, Budapest, 2003. dec. 5.

6. **Komka K**.: Gyógyszerkészítmények stabilitásvizsgálatának statisztikai kérdései, *EOQ MNB Statisztikai Módszerek Szakbizottság ülése*, Budapest, 2003. dec. 2.

7. **Komka K.**, Gyöngyösi D., Kemény S.: Az analízis időpontjának figyelembevétele gyógyszerkészítmények stabilitásvizsgálatának statisztikai értékelésénél, *Műszaki Kémiai Napok'03*, Veszprém, 2003. ápr. 8-10.

8. **Komka K.**, Kemény S.: Gyógyszerkészítmények stabilitásvizsgálatának statisztikai értékelése - problémák és továbbfejlesztési lehetőségek -, *VI. Magyar Biometriai és Biomatematikai Konferencia*, Budapest, 2002. aug. 26-27.

9. **Komka K.**, Kemény S.: Hosszútávú analitikai vizsgálatok statisztikai megközelítése, *Műszaki Kémiai Napok'02*, Veszprém, 2002. ápr. 16-18.

10. **Komka K.**: A stabilitásvizsgálat statisztikai értékelésének továbbfejlesztési lehetőségei, *Fiatal Kémikusok Előadóülése az MKE Analitikai Osztályának Szervesanalitikai Szakcsoportja rendezésében*, Budapest, 2001. nov. 14.

11. **Komka K.**, Kemény S.: A stabilitásvizsgálat statisztikai problémái, *Műszaki Kémiai Napok'01*, Veszprém, 2001. ápr. 24-26.

Other publications connected to the PhD research work

Co-author of book

Kemény Sándor - Deák András - **Lakné Komka Kinga** - Vágó Emese: Statisztikai elemzés a STATISTICA programmal, Műegyetemi Kiadó, Budapest, 2004

Oral lectures

1. **Komka K.**: Kísérlettervezés Statistica programcsomaggal, a *MTA SZAB Kemometria és Molekulamodellezés Munkabizottsága, az MKE QSAR és Modellezési Szakcsoport és a Magyar TQM Szövetség közös tudományos ülése*, Szeged, 2003. ápr. 24-25.

2. **Komka K.**, Kemény S.: Bioanalitikai módszerek validálása: a kalibráció statisztikai problémái, *Kemometria'02 Konferencia*, Tata, 2002. szept. 29. - okt. 1.

3. Kemény S., Drégelyi-Kiss Á. **Komka K.**, Deák A.: Módszerátadás statisztikai problémái, *Kemometria'01 Konferencia*, Pécs, 2001. okt. 3-5.