BUDAPEST UNIVERSITY OF TECHNOLOGY AND ECONOMICS
DEPARTMENT OF INORGANIC AND ANALYTICAL CHEMISTRY

The role of liquid chromatographic modeling software in Quality by Design principles

Thesis

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Budapest, 2015

Introduction

In the last decade, an important technical evolution has occurred in pharmaceutical analysis with numerous innovative supports and advanced instruments that have been proposed to achieve fast or ultra-fast separations in liquid chromatography (*LC*) with an excellent sensitivity and ease of operation. Among the proposed strategies to increase the throughput, the use of short narrow-bore columns packed with sub-3 μm core-shell and porous sub-2 μm particles have emerged as the gold standards. Moreover method development is also of great importance, since many cases it is the most time consuming step of drug development.

The profiling of impurities and degradation products of pharmaceuticals (*API*) is one of the most challenging tasks in liquid chromatography (*HPLC*), because of the requirements for both high-resolution and trace analysis, in addition to stringent regulatory and reporting guidelines. Finding the appropriate chromatographic conditions and then transferring the method is often challenging since they are influenced by many experimental parameters. As a consequence, the time and cost needs are high. It may happen that the method can not be transferred directly from one laboratory to another or using an alternative column is often problematic. The reasons are that selectivity and separation in *LC* depend on various parameters. Furthermore interactions can occur among these parameters.

One of the most important requirements of modern liquid chromatography in pharamaceutical develeopment is that analysis time has to be as short as possible to improve analytical throughput. In the dissertation, I focused on the theoretical and practical aspects of new column technologies namely the sub-2 μm and core-shell stationary phases. Furthermore attention has been paid to the devices that are necessary for the effective operation of these state-of-the-art columns and to the application of experimental designs and modeling software (DryLab) for method development.

Computer modelling softwares can be applied to improve the throughput as well as maximize information about method selectivity during the method development process. *HPLC* method development, robustness and Quality by Design (*QbD*) are playing an important role in the global economy, where pharmaceutical and chemical products are distributed worldwide and the method transfer process has been running for the same product in different countries and in different laboratories. Regulatory authorities (FDA, ICH, EMA, etc.) nowadays are promoting and requesting the applica-

tion of *QbD* principles to ease the exchange of complex information about chromatographic selectivity and resolution to support method control, including method development, transfer and robustness testing. By applying *QbD* approaches, a better understanding and tuning of the method can be performed to ensure a requested separation in a Design Space (*DS*). This *QbD* concept has to be applied for in-process control including old conventional high performance liquid chromatographic (*HPLC*) and as well as state-of-the-art ultra-highpressure liquid chromatographic (*UHPLC*) separations.

The goal of this work was to elaborate a liquid chromatographic method development strategy applying the combination of *UHPLC* technique and DryLab software, which enables a fast and effective method development procedure, method transfer and robustness testing. In addition it takes the *QbD* principles into consideration for the experimental designs and result assessment. My further aim was to solve some existing problems in practical liquid chromatographic separations such as the finding of alternative stationary phases (columns) for method transfer - using different devices and column geometry - to perform similar selectivity.

Theoretical background

The small extra-column volume of *UHPLC* devices allows the significant reduction of the column size. Nowdays it is common practice to use 50 x 2.1 mm columns packed with sub-2 μm core-shell particles. This reduction of column dimension involves the reduction of the analysis time. In conventional *HPLC* practice the column length varied between 100 and 250 mm. Accordingly the analysis time ranged between 10 minutes and few hours. As long as the column lengths are reduced down to 20 - 50 mm, the analysis time can significantly be decreased. Due to the shortened analysis time, the criterion for rapidity is fulfilled however it is associated with the reduction of resolution since the kinetic efficiency (theoretical plate height, *H*) and therefore the achievable theoretical plate number (*N*) depend on the column length. In order to keep the separation quality unchanged, the kinteic efficiency of small columns must be increase. One common solution to increase kinetic efficiency is to decrease the particle size [*1*].

In the literature, the small particle size is often emphasised to improve efficiency but the fact that extra-column volume is directly linked with speed and efficiency is rarely discussed. [*2*].

Another issue that is related to the extra-column volume is the system dwell time/volume. Nowdays most of the separations are carried out in the gradient elution mode. The dwell volume (V_D) has a significant impact on gradient separations, and mostly depends on the applied pump system.

During method transfer the differences between devices and column dimensions can cause serious issues. It is a common problem when traditional *HPLC* methods have to be transferred into *UHPLC* method or conversely. The extra-column volumes of the devices must be taken into account during method transfer [*3*].

The ability of a chromatographic method to successfully separate, identify and quantitate species is determined by many factors, many of which are in the control of the experimenter. When attempting to discover the important factors and then optimise a response by tuning these factors, experimental design (design of experiments, *DoE*) gives a powerful suite of statistical methodology. Advantages include modelling by empirical functions, not requiring detailed knowledge of the underlying physico-chemical properties of the system, a defined number of experiments to be performed, and available software to accomplish the task [*4,5*].

The Q8(R2) and Q11 guideline of International Conference on Harmonization (ICH) clearly define the directions for *API* and prdoduct development [*5*]. This means that during production and process controling each parameter that can affect the results must be predicted.

Modeling in liquid chromatography has begun in 1986 when DryLab appeared, which calculated retention times (t_R), retention factors (k) and critical resolution ($R_{S,crit}$) in one dimensional computing. After twenty-five years, now it is possible to predict the effect of three measured and eight calculated variables on the separation. This three dimensional model is often called as the „*cube*" [*6*]. The retention modeling of DryLab is mostly based on the solvophobic theory developed by Csaba Horváth and the linear solvent strength model. These theories explain the importance and role of water in reversed phase conditions [*7*].

The setup of the DryLab *cube* is (*Figure 1.*) the first step of model based experimental design. The independent variables - in most cases - are the temparature (T), the gradient time (t_G), and the mobile phase *pH* or the composition of ternary mixture (t_C). Two types of cube can be prepared. The t_G-*T*-*pH cube* gives information about the *pH* dependence of the components' retetnion while the influence of terner mixture on the selectivity and achievable resolution can be examined with the t_G-*T*-t_C *cube*.

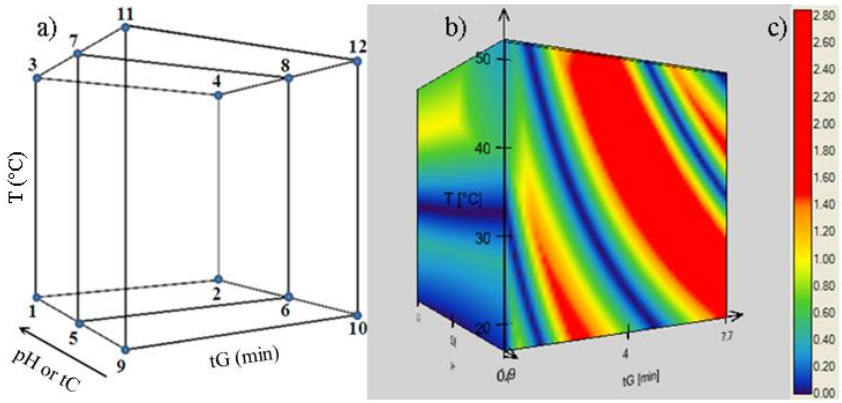

Figure 1.: a) The structure of the model cube (DoE), where the points symbolize the experimental chromatograms. b),c) The color codes show ranges where the critical resolution remains unchanged.

The circles on the corners and edges on *Figure 1. a)* indicate the experimentally measured points. The number 1, 5, 9, 3, 7 and 11 points indicate the short while the 2, 6, 10, 4, 8 and 12 points the long gradient time (t_G) – corresponding to the two level of gradient steepness. The number 1, 5, 9, 2, 6 and 10 points belong to the low while number 3, 7, 11, 4, 8 and 12 points to the high temperature value (T) – corresponding to the two level of mobile phase temperature.

Three t_G-T planars belong to the mobile phase with three different *pH* values or ternary compositions (t_C) – these variables are studied at three levels. The t_G-T planars with the same *pH* /t_C are the following points: (1, 2, 3 and 4); (5, 6, 7 and 8); (9, 10, 11 and 12).

The software calculates 97 more t_G-T planars beside the 3 measured planars so the *cube* becomes complete (*Figure 1. b)*). In the model, a chromatogram can be placed to any points, so ~100 points can imagine in a dimension, which means that in a three dimensional model with 12 measurements ~10^6 chromatograms can be simulated. The red zones in the Design Space (*DS*) indicate the points that suitable for the separation with the appropriate resolution criterion (usually R_S > 1.5). On *Figure 1. c)* the color codes help finding the appropriate conditions in the *cube* that fulfill the resolution requirement.

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[2] D. V. McCally, U. D. Neue, *J. Chromatogr. A*, **2008**, *1192*, 225

[3] A. P. Schellinger, P. W. Carr, *J. Chromatogr. A*, **2005**, *1077*, 110.

[4] D. B. Hibbert, *J. Chromatogr. B*, **2012**, *910*, 2.

[5] ICH Guidance for industry *Q8(R2)*, **2009**, *Q11*, **2012**

[6] I. Molnár, K. E. Monks, *Chromatographia*, **2011**, *73*, 5

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Experimental methods

The solvents used for the experiments were highly purified *HPLC* grade solvents. *Table 1*. contains the applied devices and their extra-column volumes. *Table 2*. includes the applied stationary phases (columns) and their most important properties. Simulations were performed using DryLab 2010 and DryLab 4 sofware.

The model compounds for method development were *API*s and their impurities being developed or marketed by Egis Phramaceutical Plc. (amlodipine, bisoprolol, loratadine and new *API*).

Table 1.: The devices and their extra column volumes used in the dissertation.

Systems	Extracolumn Volume (µL)	Dwell-Volume (mL)
Acquity UPLC	13	0.12
Acquity UPLC I-Class	8	0.1
Acquity UPLC H-Class	12	0.4
Alliance e2695 HPLC	30	1.0

Table2.: The properties of the columns used in the dissertation.

Phases	Company	Lenght (mm)	I.D. (mm)	Particle Size (µm)	Particle Type
Acquity BEH C18	Waters	50	2.1	1.7	Hybrid
Acquity BEH Shield RP 18	Waters	50	2.1	1.7	Hybrid
Acquity BEH C8	Waters	50	2.1	1.7	Hybrid
Acquity BEH Phenyl	Waters	50	2.1	1.7	Hybrid
Acquity CSH C18	Waters	50	2.1	1.7	Hybrid
Acquity CSH Phenyl-Hexyl	Waters	50	2.1	1.7	Hybrid
Acquity CSH Fluoro-Phenyl	Waters	50	2.1	1.7	Hybrid
Acquity HSS C18	Waters	50	2.1	1.8	Fully Porous
Acquity HSS C18 SB	Waters	50	2.1	1.8	Fully Porous
Acquity HSS T3	Waters	50	2.1	1.8	Fully Porous
Acquity HSS PFP	Waters	50	2.1	1.8	Fully Porous
Acquity HSS CN	Waters	50	2.1	1.8	Fully Porous
Triart C18	YMC	50	2.0	1.9	Hybrid
Cortecs C18	Waters	50	2.1	1.6	Core-Shell
Cortecs C18+	Waters	50	2.1	1.6	Core-Shell
Aeris PEPTIDE XB-C18	Phenomenex	50	2.1	1.7	Core-Shell
Kinetex XB-C18	Phenomenex	50	2.1	1.7	Core-Shell
Kinetex C18	Phenomenex	50	2.1	1.3	Core-Shell
Kinetex C18	Phenomenex	50	2.1	1.7	Core-Shell
Kinetex C18	Phenomenex	50	2.1	2.6	Core-Shell
Kinetex C18	Phenomenex	100	3	2.6	Core-Shell
Kinetex C18	Phenomenex	150	4.6	5	Core-Shell
Kinetex C8	Phenomenex	50	2.1	1.7	Core-Shell
Kinetex Phenyl-Hexyl	Phenomenex	50	2.1	1.7	Core-Shell
Kinetex PFP	Phenomenex	50	2.1	1.7	Core-Shell
Zorbax SB-C18	Agilent	50	2.1	1.8	Fully Porous
Zorbax SB-C8	Agilent	50	2.1	1.8	Fully Porous
Zorbax SB-Phenyl	Agilent	50	2.1	1.8	Fully Porous
Hypersil GOLD C18	Thermo	50	2.1	1.9	Fully Porous
Hypersil GOLD C8	Thermo	50	2.1	1.9	Fully Porous
Hypersil GOLD CN	Thermo	50	2.1	1.9	Fully Porous

Results

The main part of my research focused on the use of DryLab modeling and optimizing software. Its gain and possibilities in method development and reliability of predicted resolution were studied. Efforts were made its implementation to every-day practice to increase analytical througput and apply *QbD* strategy in the field of pharmaceutical development. Representative model compounds were selected (amlodipine and bisoprolol active pharmaceutical ingredients and their impurities from the European Pharmacopoeia (*Ph.Eur.*) to show the advantages of computer assisted method development. Besides method development a so called „simulated robustness testing" approach was also applied. The analysis time of conventional pharmacopeia methods were drastically shortened (down to the analysis time range of 5-7 minutes). The verification of prediction accuracy showed excellent agreement between calculated and measured retention times and resolutions. The *cube* used for the design and experimental results are shown in *Figure 2*. [*A*].

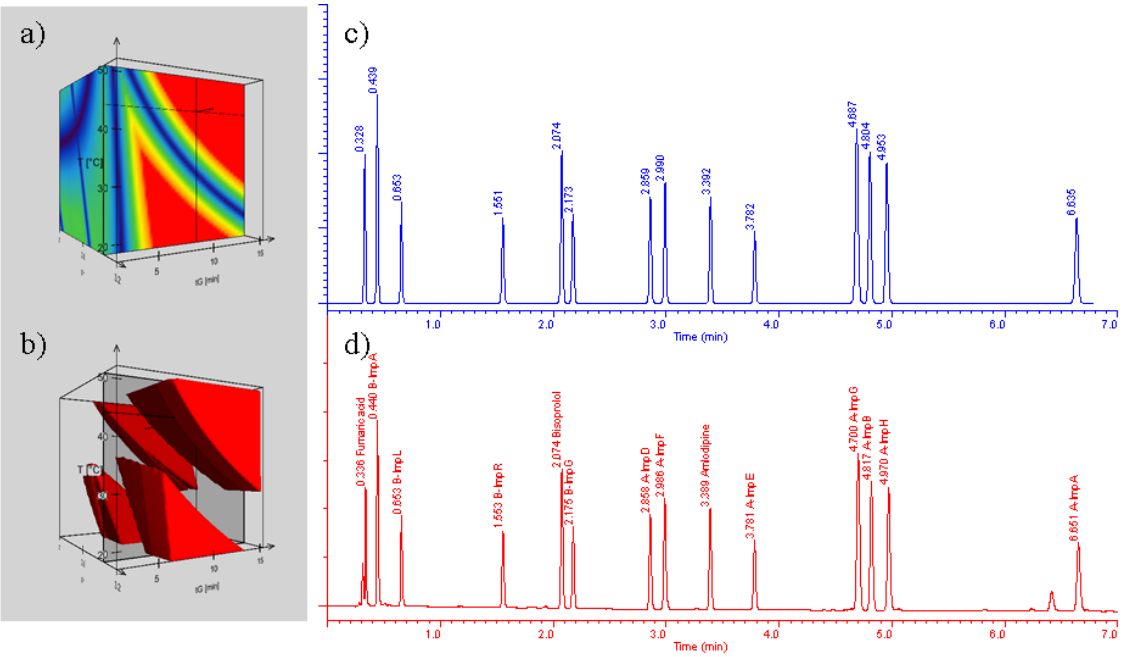
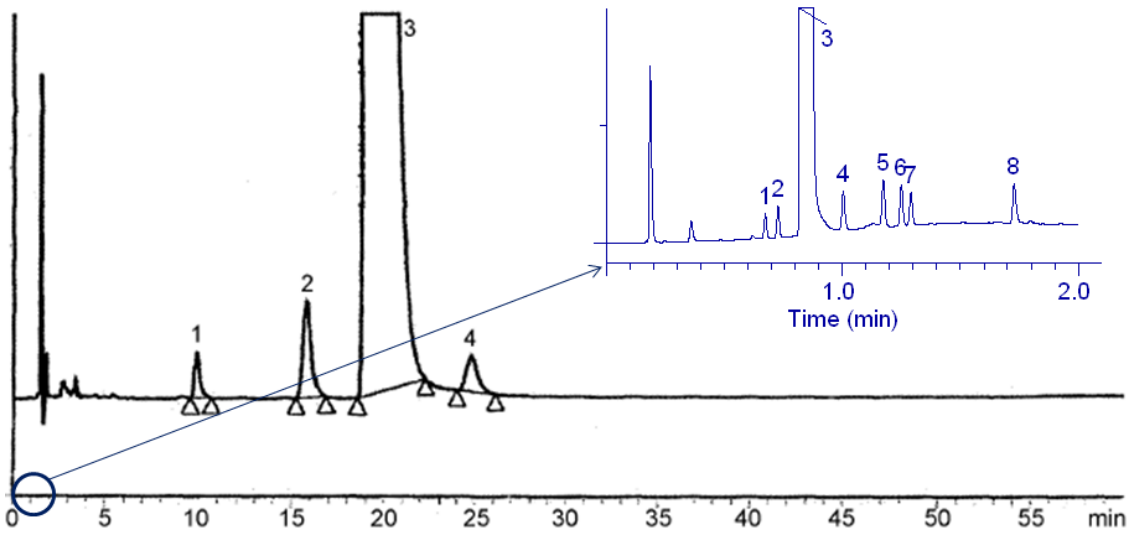


Figure 2.: a) DryLab model b) DryLab robust region of design space c) simulated chromatogram and d) experimentally observed chromatogram.

After the demonstration of the efficacy of the simulation, I proposed the same three dimensional model (based on 12 experiments) for columns of 27 different surface modifications to see the possibilities of a given separation on several stationary phases. All the columns were 50 x 2.1 mm packed with sub 2-µm particles (*Table 2*.). The

model compounds were the amlodipine and its impurities from *Ph.Eur.* An optimum (working point) for each 27 stationary phase was found suggesting that the separation is feasible whatever the stationary phase. Under the conditions suggested by the working points, the prediction accuracy was studied by the experimental verification of the measurements. The average difference between the predicetd and experimental retention times was not more than 0.04 minutes (for 6 minutes long separations). It was established that the surface modification does not affect the efficacy of the simulation. The retention behavior could be well described by the common *LSS* models. After the demonstration of the applicability of computer modeling I focused on its practical usefulness. Different *LC* separation issues have been solved by computer assisted method development.

1. The method for the separation of amlodipine and its impurities in *Ph.Eur.* is 60 minutes long and only three impurities are specified. Using the three dimensional DryLab model with the combination of *UHPLC* technology and taking the advantage of a ternary mixture, it was possible to reduce the analysis time to only 2 minutes (*Figure 3.*). The method is suitable for the separation of seven impurities beside the *API*. Its pratical significance is that 20 times more injections (taking the injection cycle time into account) can be performed within the same time [*E*].



Time (min)

Figure 3.: The chromatogram in Ph.Eur. (black) and made with QbD principle (blue). The retention orde: ImpD, ImpF, amlodipin, ImpE, ImpG, ImpB, ImpH, ImpA

2. In pharmaceutical industry, for quality control (*QC*) methods it is preferred to suggest a substiute column as an „alternative column" - for cases when the quality of the primary column changes (e.g. new batch) - which provides the same quality of separation. Thanks to modeling software, it was possible to find suitable separation ($R_{s,crit}$ > 1.5) for several tested columns, by proper adjustments of the gradient program, temperature and *pH*, while maintaining analysis time lower than 10 minutes.

The Hypersil GOLD column was found to be appropriate for substituting the reference Acquity BEH C18 column while an Acquity HSS C18 column has not fulfilled the criterion for resolution [*D*] (*Figure 4.*).

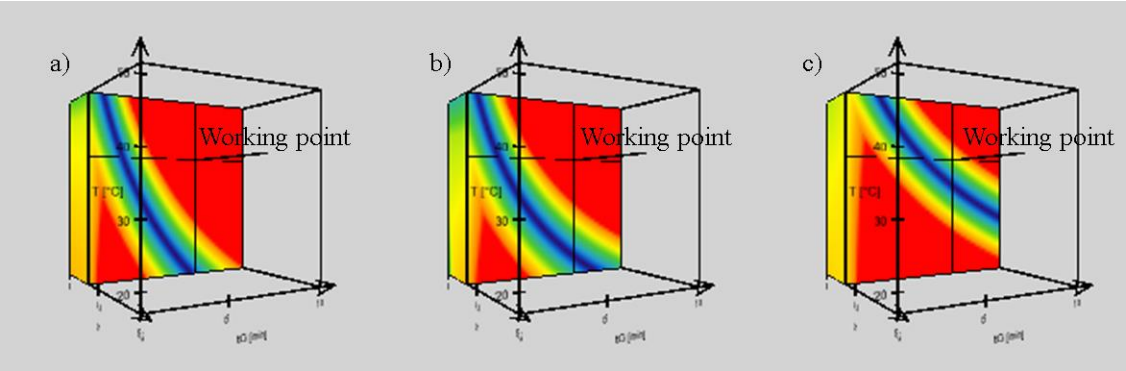


Figure 4.: DryLab model with different columns.
Acquity BEH C18 (a), Hypersil GOLD (b), Acquity HSS C18 (c)

3. During LC method transfer the different devices and columns often cause so called „out of specification" (*OOS*) results. The simulated method transfer provides a good solution for this issue.

In a first instance, the possibilities offered by modelling software for performing robustness testing were systematically compared to experimental measurements and *DoE* based predictions. The reliability of predicted retention times and resolutions were compared for the working point and at the four edges of the design space. Based on these observations, it appeared that the prediction reliability was satisfactory and then, robustness could be investigated in the early stage of method development, without generating an unacceptable amount of work for the analyst.

This confirms that modelling software is an important tool for chromatographists, to expedite method development but also robustness testing.

As result, method was successfully transferred between 50 x 2.1 mm, 100 x 3 mm and 150 x 4.6 mm columns using different *LC* systems (*Figure 5.*). *Table 1.* contains the features of the devices while the parameters of the columns are listed in *Table 2.* Loratadine and its *Ph.Eur.* impurities were selected as model compounds [*C*].

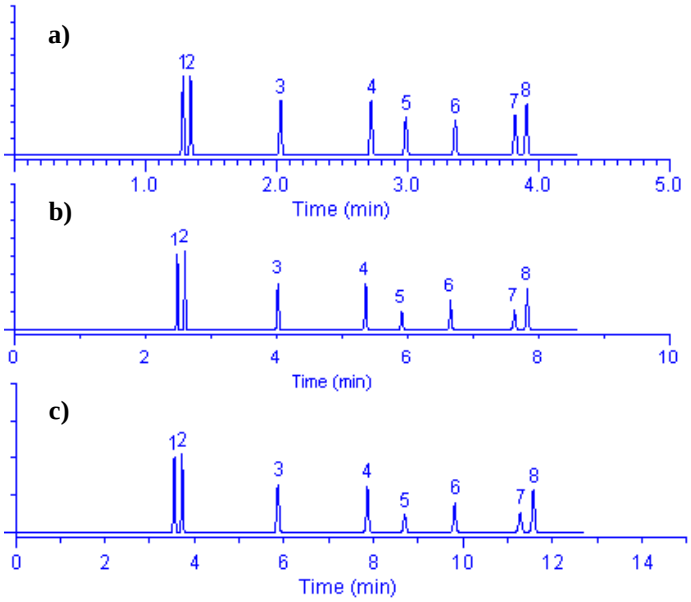


Figure 5: Simulated method transfer.
Retention order: ImpD, ImpG, ImpB, loratadin, ImpE, ImpF, ImpA, ImpC
a) Device: Acquity UPLC I-Class / Állófázis: 50 x 2,1 mm Kinetex C18, 1,7 µm
b) Device: Acquity UPLC H-Class / Állófázis: 100 x 3 mm Kinetex C18, 2,6 µm
c) Device: Alliance 2965 HPLC / Állófázis: 150 x 4,6 mm Kinetex C18, 5 µm

4. Only fast, effective and reliable analytical methods can support the work of the modern preparative reserach laboratories. To find the most influental parameters during method development an extended *pH* and temperature dependence should be tested. In these simulated models the experimental points of the *cube* are overlaped. Six *DoE cube*s were proposed where the temperature range was set as 60 °C (20°C – 80°C), the *pH* range was set 3.6 (*pH*=2,8–6,4) the gradient times were set as 1.5 and 4.5 minutes. The model compounds were the starting materials (*Stm*), intermediates (*Int*) and degradation products (*Imp*) of a synthetic route of a new *API*. The method is applicable not only for the final qualification but for the examination of the compounds in the synthetic route [*B*] (*Figure 6.*).

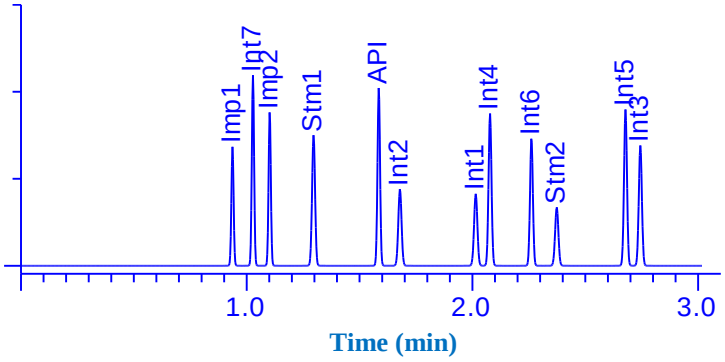


Figure 6.: Simulated chromatogram for the sparation of API and its impurities.

Thesis points

1. We first published on the comparison of simulated and experimentally measured robustness testing. A main advantage of the simulated robustness test is that it takes into account the interactions of the model parameters. This confirms that modelling software is an important tool for chromatographists, to expedite method development but also robustness testing. 27 different types of stationary phases have been tested with the same model. The difference between the simulated and measured retention times was not higher than 0.03 minutes, which means less than 1% relative error in retention time prediction [*A*, *F*].

2. I developed a fast liquid chromatographic method (total run time is 2 minutes) using *QbD* principle for the separation of amlodipine and its impurities. The European Pharmacopeia suggests a 60 minutes long separation. Considering the time of injection cycle, the throughput can be improved by a factor of 20 [*E*].

3. A simulated column substituability test was applied for *LC* separations. Using this strategy it can easily be decided if two different columns can replace each other under the given chromatographic coditions or not [*D*].

4. A simulated method transfer was succesfully applied in *LC* practice without the need of real experiments. The model hepls to foresee the transferred chromatogram, which makes the analytical method transfer easier and faster between different devices and column dimensions [*C*].

5. I evolved a fast and robust liquid chromatographic method development approach for the full investigation of the synthesis of *API*s. The time required for an „extended" method development (including extended *pH* and temperature range) is about only 1 day. The final analysis time is few minutes so the method can be applied for both *QC* and preparative research laboratories for during in-process control [*B*].

Application

The development strategies finding in the results and thesis points are applied successfully in analytical R&D laboratories of Egis Pharmaceutical Plc.

Publications and presentations regarding the dissertation

Journal articles

[*A*] R. Kormány, I. Molnár, J. Fekete, D. Guillarme, Sz. Fekete, *Chromatographia*, **2014**, *77*, 1119-1127
Impact Factor: 1,37 (2013/2014)
Citation: -

[*B*] R. Kormány, I. Molnár, J. Fekete, *LCGC North America*, **2014**, *32/5*, 354-363, *LCGC Europe*, **2014**, *27/5*, 240-248
Impact Factor: *LCGC North America*: 0,356 (2013/2014)
LCGC Europe: 0,655 (2013/2014)
Citation: 1

[*C*] R. Kormány, J. Fekete. D. Guillarme, Sz. Fekete, *J. Pharm. Biomed. Anal.*, **2014**, *94*, 188-195
Impact Factor: 2,829 (2013/2014)
Citations: 2

[*D*] R. Kormány, J. Fekete, D. Guillarme, Sz. Fekete, *J. Pharm. Biomed. Anal.*, **2014**, *89*, 67-75
Impact Factor: 2,829 (2013/2014)
Citations: 4

[*E*] R. Kormány, H.-J. Rieger, I. Molnár, *LCGC North America*, **2013**, *31/S4a*, 2-8, *LCGC Europe*, **2013**, *26/10/Suppl.*, 14-19
Impact Factor: *LCGC North America*: 0,356 (2013/2014)
LCGC Europe: 0,655 (2013/2014)
Citation: 1

[*F*] R. Kormány, I. Molnár, H.-J. Rieger, *J. Pharm. Biomed. Anal.*, **2013**, *80*, 79-88
Impact Factor: 2,829 (2013/2014)
Citations: 5

Book

A folyadékkromatográfia fejlesztési irányai, gyors folyadékkromatográfia
Fekete Jenő, Kormány Róbert, Fekete Szabolcs
Merck Kft., **2014** ISBN 978 963 08 9407 4

Presentations

Folyadékkromatográfiás módszerek szimulált tervezése
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DryLab 3-dimenziós modell alkalmazási lehetőségei
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XLIV. Kromatográfiás Továbbképző Tanfolyam, Szeged, 2013.01.28-30

Folyadékkromatográfiás módszerfejlesztés számítógépes szimulációval
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Elválasztástudományi Vándorgyűlés 2012, Hajdúszoboszló, 2012.11.07-09

Posters

Robust UHPLC Method Development Using Computer Modeling
Róbert Kormány, Imre Molnár, Jenő Fekete
9th Balaton Symposium, 2013.09.04-06

Quality by Design in UHPLC Method Development
Róbert Kormány, Imre Molnár, Hans-Jürgen Rieger
HPLC2013 Amsterdam, 2013.06.16-20