Technical paper

Experimental Determination of Numerical Values of Critical Parameters in the Gatifloxacin Process Development

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Abstract

A practical example of the determination of critical parameters is presented on the example of the gatifloxacin process development.

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1. Introduction

The knowledge of critical parameters is a very important aspect in the management and control of chemical processes. Recently, a general definition of numerical determination of critical parameters in chemical processes was presented and, in this article, we would like to show a practical example of the determination of critical parameters during the chemical process development of gatifloxacin ((±)-1-cyclopropyl-6-fluoro-1,4-dihydro-8-methoxy-7-(3-methyl-1-piperazinyl)-4-oxo-3-quinoline carboxylic acid sesquihydrate).[1] This process was improved at some stages and published in a patent application in 2006.[2]

Gatifloxacin was discovered by the company Kyorin and is a synthetic broad-spectrum 8-methoxyfluoroquinolon antibacterial agent (DNA girase inhibitor) for oral or intravenous administration.[3] In vitro, it is active against gram-positive and gram-negative aerobic bacteria. At the beginning it was supposed to be very promising and to have few adverse effects.[4] However, in the past year the product Tequin, containing the active substance gatifloxacin, was withdrawn from the marked due to life-threatening adverse effects (dysglycemia).[5]

The general definition of numerical determination of critical parameters is presented by the expression:

$$P(sp) := o(I_{working}, y), \quad (1)$$

Where:
P – working parameter,
sp – set point,
o – symbol of the (none)criticalness,
$I_{working}$ – working interval,
y – factor of criticalness,
:= – an assignment sign.

or in more specific examples (time-dependent working parameters):

$$P(sp) := o(I_{working}, y, z), \quad (2)$$

Where:
P, sp, o, $I_{working}$ and y are the same as for the basic definition
z – relative time portion of some operation

The above definitions are especially useful in cases where the time dependence of the factor of criticalness and the size of the working interval between two process time points are constant especially for time-dependent process parameters (e.g. T).

If the size of the working interval and the factor of criticalness are time-dependent, the general definition is as follows:

$$P(sp) := o(I_{working}(z), y(z), z), \quad (3)$$

P, sp, o and z are the same as in the basic definition
$I_{working}(z)$, y(z) – time (fraction in the process operation) dependent functions $I_{working}$ and y.

For the sake of simplicity, we tend to choose such observation parameters where the expressions (1) and (2) are preferentially used, providing that the correctness and accuracy of the chemical process description with these expressions are met.

Substances that are used as active pharmaceutical ingredients (API) must fulfil specific regulatory quality requirements, published in a Pharmacopoeia (Ph. Eur., USP, etc.) and International Conference on Harmonisation (ICH) Guidelines. These quality requirements (such as those relating to purity, assay, level of residual solvents) depend on the daily dose level of the medicine and are calculated on the maximum allowed daily dose of the medicine.

In general, for every compound the medicine contains (API, intermediates and other ingredients) and is used in the chemical synthesis of the API, a detailed analytical specification must or should be made. With this specification, which should include optimal number of analytical tests, the quality of a specific substance (API, intermediate) is very well controlled and determined. In case of a new drug application (NDA) or an application for a new version of a generic product (ANDA), these specifications have to be substantiated with evidence before regulatory authorities (FDA, EMEA).

2. Experimental

The improved chemical synthesis process of gatifloxacin sesquihydrate was developed in Krka using two computer-controlled systems. For screening we used the parallel system Surveyor of the company Argonaut (today BioTage) and for optimization, validation and determination of critical parameters, the LARS system (Laboratory Reactor System, 1 l reactor), which was developed in Krka in cooperation with the company BIA.[6,7]

The specification of the final sample of gatifloxacin sesquihydrate includes appearance of the compound, solubility in solvents (acetic acid, DMF, acetone, methanol), identification (IR, UV), degree of coloration of the solution, clarity and degree of opalescence of the solution, heavy metals, residue on ignition, water content (K.F.), residual solvents, specific optical rotation, sulphate ash, water assay, related substances (individual, total), assay (potentiometric, HPLC) and other internal for formulation important tests.

Most of the tests are performed according the Ph. Eur. The HPLC analysis is performed according the HPLC method published in Krka's patent application.

During the process development we used starting materials, reagents and solvents, for which the specifications were set and defined. Based on experiments, isolations of impurities, other possible identifications of other impurities (LC-MS) or on independent syntheses of the same, carry over of each impurity to subsequent phases was determined.

During the development phase the following impurities were identified in the final product or in synthesis of the intermediates.

1. 1-Cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (starting material, GPX60)

Conditions for appearance: if reaction is not 100% completed due to too short reaction time, remainings are presented in the crude gatifloxacin base.

2. (±) 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (intermediate, GPX62)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase and is presented in the final product when the phase of formation gatifloxacin sesquihydrate is not completed.

3. (±) Ethyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (impurity, GPX63)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase from impurity GPX84, which could be present in starting material GPX60.

4. (±) Ethyl 1-cyclopropyl-6-fluoro-8-hydroxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (impurity, GPX64)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as degradation product GPX63.

5. (±) 1-Cyclopropyl-6-fluoro-8-hydroxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX65)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as degradation product of GPX62 or as a product between 2-methyl piperazine and GPX77.

6. (±) Methyl 1-cyclopropyl-6-fluoro-8-hydroxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (impurity, GPX66)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as degradation product of GPX69.

7. (±) 1-Cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hemihydrate (impurity, GPX67)

Conditions for appearance: it is synthesized in the phase of formation of gatifloxacin sesquihydrate due to wrong synthesis conditions.

8. (±) Methyl 1-cyclopropyl-6-fluoro-8-methoxy-7-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate (impurity, GPX69)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as synthesis by-product between GPX85, which could be present in GPX60 and 2-methyl piperazine.

9. 7-(2-Aminoethylamino)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX72)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as degradation product of GPX62 or as synthesis by-product between GPX60 and ethylenediamine, which could be present in 2-methyl piperazine.

10. 7-(2-Aminopropylamino)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX73)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as degradation product of GPX62 or as synthesis by-product between GPX60 and 1,2-diamino propane, which could be present in 2-methyl piperazine.

11. 7-(1-Aminopropan-2-ylamino)-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX74)

Conditions for appearance: it is synthesized in the nucleophilic reaction phase as degradation product of GPX62 or as synthesis by-product between GPX60 and 1,2-diamino propane, which could be present in 2-methyl piperazine.

12. 7-Chloro-1-cyclopropyl-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid, (impurity, GPX75)

Conditions for appearance: it is an impurity in the starting material GPX60.

13. 1-Cyclopropyl-6-fluoro-8-methoxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX76)

Conditions for appearance: it is synthesized in the nucleophilic reaction as by-product between GPX60 and piperazine, which could be present in 2-methyl piperazine.

14. 1-Cyclopropyl-6,7-difluoro-8-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX77)

Conditions for appearance: it is synthesized in the nucleophilic reaction as by-product between GPX60 and piperazine, which could be present in 2-methyl piperazine.

15. 1-Cyclopropyl-6-fluoro-7-hydroxy-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX81)

Conditions for appearance: it is synthesized in the nucleophilic reaction as by-product from GPX60.

16. Ethyl 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (impurity, GPX84)

Conditions for appearance: it is an impurity in the starting material GPX60.

17. Methyl 1-cyclopropyl-6,7-difluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylate (impurity, GPX85)

Conditions for appearance: it is an impurity in the starting material GPX60.

18. (±) 1-Cyclopropyl-7-(3,5-dimethylpiperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX87)

Conditions for appearance: it is synthesized in the nucleophilic reaction as by-product between GPX60 and (±)-2,6-dimethyl piperazine, which could be present in 2-methyl piperazine.

19. (±)-1-Cyclopropyl-7-(3,5-dimethylpiperazin-1-yl)-6-fluoro-8-hydroxy-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX88)

Conditions for appearance: it is synthesized in the nucleophilic reaction as degradation product of GPX87.

20. 1-Cyclopropyl-6-fluoro-8-hydroxy-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPX90)

Conditions for appearance: it is synthesized in the nucleophilic reaction as degradation product of GPX76.

21. (±) 1-Cyclopropyl-7-fluoro-8-methoxy-4-oxo-6-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPXBA)

Conditions for appearance: it is synthesized in the nucleophilic reaction as by-product between GPX75 and 2-methyl piperazine.

22. (±) 1-Cyclopropyl-7-fluoro-8-methoxy-6-(3-methylpiperazin-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (impurity, GPXBB)

Conditions for appearance: it is synthesized in the nucleophilic reaction.

Both enantiomeric forms of gatifloxacin sesquihydrate were prepared.

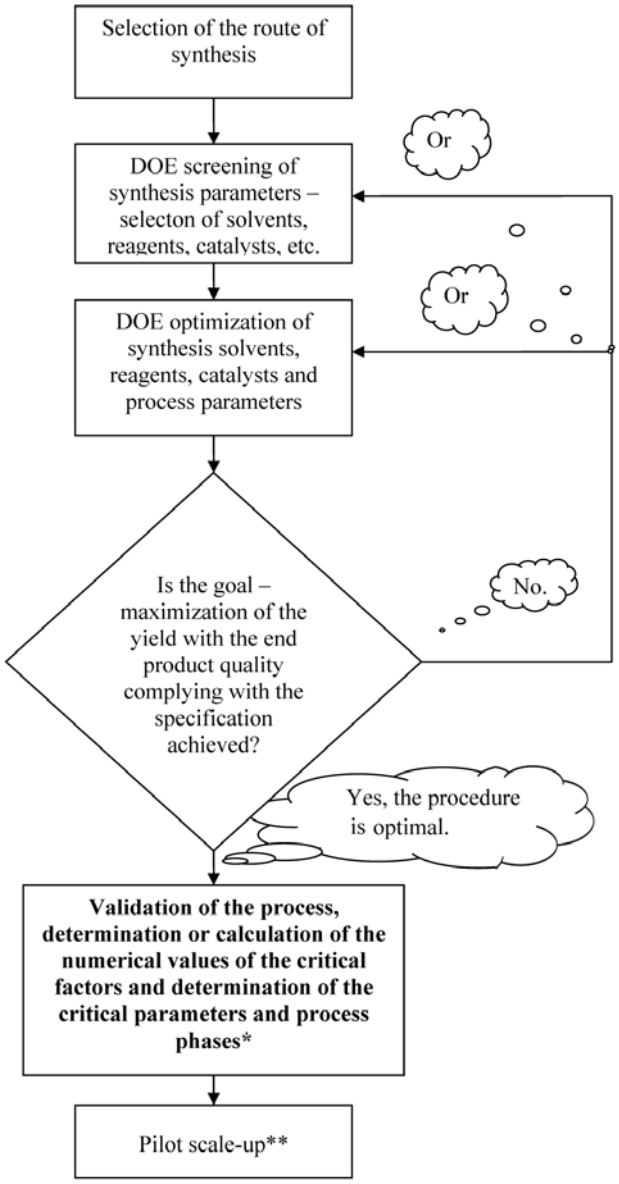
The development phase of the synthesis was first carried out on the parallel system Surveyor (scale 1 to 2 g), where screening of the solvent and reaction conditions, purification steps and preparation of sesquihydrate were performed. The DOE (Design of Experiments) screening method was used for this phase[8, 9]. In the phase of screening, already patented solutions for synthesis and purification had to be considered.[3, 10–2]

After optimal solvents and rough synthesis conditions were selected, the LARS system was used for the optimization and validation of these conditions on the scale from 10 g to 100 g, depending on the synthesis phase. DOE optimisation methods (multivariate analysis like full or fractional factorial, Plackett Burman, D-Optimal, Box Behnken, etc designs) were used and dependence functions between outcome process parameters and incoming working variables were determined. Most of these functions were first grade order.

The major part of experimentation was carried out in the screening part where in the most cases 2 levels fractional factorial method was used. In that part of experimentation for each of the key outcome parameter (dependant working parameter, list of them is on the next page) the group of the most importat working parameters was determined according to values of the scaled and centered coeffieient from the model for each of them.

The number of the experiments in one of our screening design for the etc. 4 key outcome parameters and 6 independent working parameters was around 20 experi-

ments for fractional factorial linear model (the number of experiments is requested by the model configuration). This number changes according to number of key outcome parameters, independent working parameters, number of levels, number of replications of the central points, etc. Analogically was in the optimization phase.



Scheme 1: the process development of the chemical synthesis for API and location (bold) of the numerical determination of critical parameters phase in it

* Factors of criticalness are mostly calculated from the optimization database (MS Access database was used for storing the data) and from certain optimization models (DOE software (etc. SAS, Stavex or any other) could be used for calculating such models, where the final result is polynomial function of output process parameters depending on independent process parameters).
** If in the scale-up procedure it is noticed that there are some differences between factors of criticalness due to the scale-up effect, factors for these parameters are calculated or determined again.

The whole number of the experiments for the screening and the optimization of the synthesis was in the range of some hundereds. Because of that the automation of the experimentation is very helpful.

Scheme 1 shows the process development of the chemical synthesis for API and location (bold) of the numerical determination of critical parameters phase in it.

We have found out that the key outcome parameters (dependant working parameters) from specifications with which the optimal and critical process conditions were determined in the phase of the synthesis and purification of the crude gatifloxacin base and the synthesis of gatifloxacin sesquihydrate are as follows:

• ***Crude gatifloxacin base:***
– impurities GPX65, GPX73, GPX76, GPX77 and GPXBB. Other properties from the final specification (such as optical rotation, heavy metals) are preferablly controled by the right selection of the incoming materials.

During the development phase of all three process steps, the levels of purification of the critical impurities under selected conditions were determined and, consequently, allowed limit values in the crude product were set. According to these levels, and together with the maximization of the yield, optimal synthesis conditions were selected.

• ***Purified gatifloxacin base***
– impurities GPX65, GPX76 and GPXBB,
– colourisation level of solution, clarity and degree of opalescence of solution,
– residual solvents (especially DMSO).

• ***Final gatifloxacine sesquihydrate***
– impurity GPX65,
– heavy metals,
– residue on ignition,
– water assay (KF) during the drying phase of the final product,
– internally specified physical properties of the product during milling.

According to the above quoted key outcome parameters, optimal synthesis conditions were selected and specifications for the In-Process Control (IPC) methods were set. The maximal allowed shift for each analyzed parameter was determined compared with the measured values for each when the optimal synthesis conditions were applied, and values for factors of criticalness were calculated there off.

It is important to stress out that factors of criticalness are determined according to the quality of the product only, whereas the yield is always maximized as possible in the selected route of synthesis.

3. Results and Discussion

In the continuation, an example of practical determination of numerical values of criticalness is given for most important parameters (the most important parameters were also empirically justified) and, due to shortage of space, just for crystallization steps. In the real laboratory and industrial procedure, numerical values for parameters were determined or recalculated for all steps.

In addition, the procedure for the virtual production line with different fitness (different working parameters) is shown. We can see how criticalness of a parameter changes as a function of the quality (fitness) of the production system (equipment).

During the industrial scale-up experiments an interesting anecdote happened on the limit point (71 °C) in the second crystallization, where conditions for the achievement of the limit point were not performed (the temperature for the allowed interval of the limit point (70–71 °C) was not reached). Because of that, the product was not dissolved completely in the crystallization phase solvent and during the hot filtration with activated charcoal, the product remained on the filter. The quality of the final product was therefore unsatisfactory; this was corrected with an additional reprocessing step for that batch.

For the crystallization step these dependant key parameters (impurities GPX65, GPX76 and GPXBB; colourisation level of solution, clarity and degree of opalescence of solution; residual solvents (especially DMSO)), as mentioned above, were used as measure for criticalness of some working process parameter. E.g. the level of the allowed solvent volume shift was determined according to the allowed level (see specification settings) of one of the impurities (GPX65, GPX76, GPXBB – the one with the highest level in the purified gatifloxacin base), colourisation level of solution or residual solvents which were determined as key outcome parameters. The same was for all independent working process parameters (T, t, V). So, if there is an expression:

$$V\downarrow(625\ ml) = C(0.03, 2.5) \quad (4),$$

that means that the allowed shift (0,03 x 625 ml x 2,5 = 47 ml; for understanding this calculation see the Ref. 1) of the solvent volume is determined according to the allowed shift (according to specification for the purified gatifloxacin base) of the level of one (the one with the highest level in the purified gatifloxacin base) impurity.

In general, level of impuritiy in API could be presented as function of independent process working parameters:

$$\text{Level of key impurity} = f(V, T, t, \ldots) \quad (5).$$

Because process is managed by the proper selection of these independent working parameters (V, T, t, …), from function f we can find the correlation which shows how, e.g.:

$$V = g(\text{level of key impurity}) \quad (6),$$

working process parameter (V) is according to specification for this intermediate (allowed level of this impurity) dependant from "level of key impurity". Most often function g is inverse function of f. Don't forget that through determination of g all except one independent working parameters are constant (see Ref. 1).

4. Real Production Equipment

4. 1. First Crystallization

125 ml demineralised water, crude wet gatifloxacin base from the previous phase and 500 ml methanol (the critical parameter is determined for the mixture of methanol and water: $V\downarrow(625\ ml) = C(0.03, 2.5)$, $V\uparrow(625\ ml) = N(0.03, 4.5)$; if we make a mistake when we prepare the mixture, it is better to have more water than methanol with regard to the prescribed ratio) are charged to the 1000 ml reactor, with stirrer, temperature probe and reflux condenser at 25 °C (20 °C – 30 °C)) and the suspension is mixed for 15 minutes. The temperature slightly increases.

Homogenized suspension is heated to the reflux – 71 °C ($T\downarrow(71\ °C) = C(2\ °C, 1)$ – the limit point is limited upwards because of reflux) in 45 minutes ($t(45\ min) = N(0.05, 5)$) and mixed at this temperature maximum for 30 minutes ($t\downarrow(30\ min) = N(0.05, 4)$, $t\uparrow(30\ min) = N(0.05, 80)$). The solution is cooled to 5 °C in 120 minutes ($t(120\ min) = N(0.05, 5)$) and mixed at 5 °C for 30 minutes ($t\downarrow(30\ min) = N(0.05, 3.5)$, $t\uparrow(30\ min) = N(0.05, 10)$. The suspension is filtered over a filter MN 640 w (black ribbon), washed with 62.5 ml mixture of methanol : water = 50 ml : 12,5 ml and throughly sucked. Filtration lasts for 3 minutes (from 2 to 5 minutes), washing and sucking last for 75 minutes (from 45 to 125 minutes).

The weight of wet product is measured, the level of residual solvent is determined and the yield of the crystallization is calculated.

4. 2. Second Crystallization

125 ml demineralised water is charged to the 1000 ml reactor, with stirrer, temperature probe and reflux condenser at 25 °C (20 °C – 30 °C). Once purified, the product from the previous phase is added together with 0.1 g EDTA and 500 ml methanol (the critical parameter is determined for the mixture of methanol and water; $V\downarrow(625\ ml) = C(0.03, 2.5)$, $V\uparrow(625\ ml) = N(0.03, 4.5)$; if we make a mistake when we prepare the mixture, it is better to have more water than methanol with regard to the prescribed ratio) and suspension is mixed for 15 minutes. The temperature slightly increases.

Homogenized suspension is heated to the reflux – 71 °C (T↓(71 °C) = C(2 °C, 0.5) – the limit point is limited upwards because of reflux) in 45 minutes (t(45 min) = N(0.05, 5)) and mixed at this temperature maximum so long that everything is dissolved – 30 minutes (t(30 min) = N(0.05, 4)). The solution must be clear before performing the filtration, which should be as fast as it can be. The filtrate is collected in the other reactor and again reheated to 71°C. Then, the solution is cooled to 5 °C in 120 minutes (t(120 min) = N(0.05, 5)) and mixed at 5 °C for 30 minutes (t↓(30 min) = N(0.05, 3.5), t↑(30 min) = N(0.05, 10). The suspension is filtered over a filter MN 640 w (black ribbon), washed with 62.5 ml demineralised water and throughly sucked. Filtration lasts for 6 minutes (from 2 to 12 minutes), washing and sucking last for 55 minutes (from 40 to 70 minutes).

The weight of wet product is measured, the level of residual solvent is determined and the yield of the crystallization is calculated.

List of the numerical determination of critical parameters for the real production equipment can be presented as it is shown in Table 1.

Virtually changed production equipment (new working intervals are bolded)

New working intervals for the virtual production equipment are made up just to show the difference, what happen with the status (critical/noncritical) of the parameters when the fitness (working intervals) of production equipment is changed.

4. 3. First Crystallization

125 ml demineralised water, crude wet gatifloxacin base from the previous phase and 500 ml methanol (critical parameter is for the mixture methanol and water; V↓(625 ml) = **N**(**0.01**, 7.5), V↑(625 ml) = **N**(**0.01**, 13.5); if we make a mistake when we prepare the mixture, it is better to have more water than methanol with regard to the prescribed ratio) are charged to the 1000 ml reactor, with stirrer, temperature probe and reflux condenser at 25 °C (20 °C – 30 °C).) and suspension is mixed for 15 minutes. The temperature slightly increases.

Homogenized suspension is heated to the reflux – 71 °C (T↓(71 °C) = **C**(**1** °C, 2) – limit point is limited upwards because of reflux) in 45 minutes (t(45 min) = **N**(**0.02**, 12.5)) and mixed at this temperature maximum for 30 minutes (t↓(30 min) = **N**(**0.02**, 10), t↑(30 min) = **N**(**0.02**, 200)). Solution is cooled to 5 °C in 120 minutes (t(120 min) = **N**(**0.02**, 12.5)) and mixed at 5 °C for 30 minutes (t↓(30 min) = **N**(**0.02**, 8.75), t↑(30 min) = **N**(**0.02**, 25). Suspension is filtered over filter MN 640 w (black ribbon), washed with 62,5 ml mixture methanol : water = 50 ml : 12,5 ml and throughly sucked. Filtration lasts for 3

Table 1: List of the numerical determination of critical parameters for real production equipment

Critical Parameter	Operation	Numerical values of the critical arameters
	FIRST CRYSTALLIZATION	
V of mixture water/methanol in the first crystallization	**Charging of the first crystallization solvent mixture**	V↓(625 ml) = C(0.03, 2.5), V↑(625 ml) = N(0.03, 4.5)
T of the first crystallization	**Conditions of the first crystallization**	71 °C (T↓(71 °C) = C(2 °C, 1)
t of heating on the T of the first crystallization		t(45 min) = N(0.05, 5)
t of dissolving of the product on the T of the first crystallization		t↓(30 min) = N(0.05, 4), t↑(30 min) = N(0.05, 80)
t of cooling on 5 °C in the first crystallization		t(120 min) = N(0.05, 5)
t of mixing at the 5 °C in the first crystallization	**Conditions of the isolation of the product in the first crystallization**	t↓(30 min) = N(0.05, 3.5), t↑(30 min) = N(0.05, 10)
	SECOND CRYSTALLIZATION	
V of mixture water/methanol in n the second crystallizatio	**Charging of the second crystallization solvent mixture**	V↓(625 ml) = C(0.03, 2.5), V↑(625 ml) = N(0.03, 4.5)
T of the second crystallization	**Conditions of the second crystallization**	71 °C (T↓(71°C) = C(2 °C, 0.5)
t of heating on the T of the second n crystallizatio		t(45 min) = N(0.05, 5)
t of dissolving of the product on the T of the second crystallization		t(30 min) = N(0.05, 4)
t of cooling on 5 °C in the second crystallization		t(120 min) = N(0.05, 5)
t of mixing at 5 °C in the second crystallization	**Conditions of the isecond of the product in the crystallization solation**	t↓(30 min) = N(0.05, 3.5), t↑(30 min) = N(0.05, 10)

minutes (from 2 to 5 minutes), washing and sucking last for 75 minutes (from 45 to 125 minutes).

Weight of wet product is measured, level of residual solvent is determined and yield of the crystallization is calculated.

4. 4. Second Crystallization

125 ml demineralised water is charged to the 1000 ml reactor, with stirrer, temperature probe and reflux condenser at 25 °C (20 °C – 30 °C). Once purified product from the previous phase is added together with 0,1 g EDTA and 500 ml methanol (critical parameter is determined for the mixture of methanol and water; V↓(625 ml) = **N(0.01**, 7.5), V↑(625 ml) = **N(0.01**, 13.5); if we make a mistake when we prepare mixture, it is better to have more water than methanol with regard to the prescribed ratio) and suspension is mixed for 15 minutes. The temperature slightly increases.

Homogenized suspension is heated to the reflux – 71 °C (T↓(71 °C) = **C(1 °C,** 1) – the limit point is limited upwards because of reflux) in 45 minutes (t(45 min) = **N(0.02,** 12.5)) and mixed at this temperature maximum so long that everything is dissolved – 30 minutes (t(30 min) = **N(0.02,** 10)). The solution must be clear before performing the filtration, which should be as fast as it can be. The filtrate is collected in the other reactor and again reheated to 71°C. Then, the solution is cooled to 5 °C in 120 minutes (t(120 min) = **N(0.02,** 12.5)) and mixed at 5 °C for 30 minutes (t↓(30 min) = **N(0.02,** 8.75), t↑(30 min) = **N(0.02,** 25). The suspension is filtered over a filter MN 640 w (black ribbon), washed with 62.5 ml demineralised water and thoroughly sucked. The filtration lasts for 6 minutes (from 2 to 12 minutes), washing and sucking last for 55 minutes (from 40 to 70 minutes).

The weight of wet product is measured, the level of residual solvent is determined and the yield of the crystallization is calculated.

List of the numerical determination of critical parameters for the virtual production equipment can be presented as it is shown in Table 2.

As it could be seen from the comparison of the status of the same parameter in Table 1 and Table 2 for the real (worse fitness, working intervals are bigger) and the virtual (better fitness, working intervals are smaller) production equipment, in the last case there are less critical parameters and process is easier to be controlled. This information is very important for the decision on which production line the process will be performed and also during the production planning when some process phases (time schedule, how many workers will be available for the specific task) will be done.

Table 2: List of the numerical determination of critical parameters for virtual production equipment

Critical Parameter	Operation	Numerical values of the critical parameters
	FIRST CRYSTALLIZATION	
V of mixture water/methanol in the first crystallization	**Charging of the first crystallization solvent mixture**	V↓(625 ml) = **N(0.01,** 7.5), V↑(625 ml) = **N(0.01,** 13.5)
T of the first crystallization t of heating on the T of the first crystallization t of dissolving of the product on the T of the first crystallization t of cooling on 5 °C in the first crystallization	**Conditions of the first crystallization**	71 °C (T↓(71 °C) = **C(1** °C, 2) t(45 min) = **N(0.02,** 12.5) t↑(30 min) = **N(0.02,** 10), t↓(30 min) = **N(0.02,** 200) t(120 min) = **N(0.02,** 12.5)
t of mixing at the 5 °C in the first crystallization	**Conditions of the isolation of the product in the first crystallization**	t↓(30 min) = **N(0.02,** 8.75), t↑(30 min) = **N(0.02,** 25)
	SECOND CRYSTALLIZATION	
V of mixture water/methanol in secondcrystallization	**Charging of the second crystallization solvent mixture**	V↓(625 ml) = **N(0.01,** 7.5), the V↑(625ml) = **N(0.01,** 13.5)
T of the second crystallization t of heating on the T of the second crystallization t of dissolving of the product on the T of the second crystallization t of cooling on 5 °C in the second crystallization	**Conditions of the second crystallization**	71 °C (T↓(71 °C) = **C(1** °C, 1) t(45 min) = **N(0.02,** 12.5) t(30 min) = **N(0.02,** 10) t(120 min) = **N(0.02,** 12.5)
t of mixing at 5 °C in the second crystallization	**Conditions of the isolation of the product in the second crystallization**	t↓(30 min) = **N(0.02,** 8.75), t↑(30 min) = **N(0.02,** 25)

5. Conclusion

The procedure for the determination of the critical parameters is presented on the example of the process development of API gatifloxacin. This procedure is an upgrade of the process of the development, optimization and validation of chemical processes and its goal is to determine the criticalness of the process, identification and numerical evaluation of critical parameters in the synthesis process. The aims of determining critical parameters are: to deepen the knowledge of chemical processes, to determine numerically the criticalness of the production process, and to achieve an easier, faster and profounder transfer of knowledge between development and production experts/workers.

Indirectly, the process for the determination of critical parameters demands thorough knowledge of the laboratory and industrial equipment, including calibration of all measuring instruments and determination of working intervals for the systems in which the processes are performed.

The chemical process which is described in this article was actually tested in industrial scale. All laboratory-determined critical factors were recalculated to the industrial values, using industrial working intervals for the selected equipment.

An illustration of recalculation of the critical parameters for an other virtual (or real) production line shows how criticalness of certain parameters changes; in some cases it changes in a way that some parameters are not critical any more and the factor of criticalness is increased far beyond 9 and could be omitted.

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7. References

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Povzetek

Predstavljen je praktičen primer določitve numeričnih vrednosti kritičnih parametrov na primeru preparata gatifloksacin.