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Research Article

Quality by design approach for simultaneous determination of original active pharmaceutical ingredient quinabut and its impurities by using HPLC. Message 1

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Abstract

Aim. The aim of study was to develop and validate a simple, highly robust (quality by design (QbD) approach), precise and accurate method using high performance liquid chromatography for the simultaneous determination of original active pharmaceutical ingredient Quinabut and its impurities.

Materials and methods. Experiments were performed on a Shimadzu LC-20 Prominence HPLC separation module, equipped with a quaternary gradient pump, temperature controlled column heater, sampler manager and diode array detector and LC-20 Chemstation for data analysis (Shimadzu Corporation, Japan). Same software was used for data acquisition and processing of results. X-Terra RP18 (4.6×150 mm, 5 μm) analytical chromatographic column provided by Waters Corporation (Milford, MA) was used for all optimization experiments. Mobile phase A: *acetonitrile R*. Mobile phase B: 0.025 M phosphate buffer solution. Samples were chromatographed in gradient mode. Flow rate of the mobile phase: 0.7 mL/min. Column temperature: 40 °C. Detection: at 233 nm wavelength. Injection volume: 50 μl.

Results. Screening of the influence of four chromatographic factors on different chromatographic responses was performed as the initial step of analytical method optimization. A randomized fractional factorial experimental design (2^{4-1}) of resolution IV with central point was used. Buffer pH, amount of acetonitrile in mobile phase A, the amount of phosphate buffer solution in mobile phase B and column temperature were selected as factors of interest, and were used to generate the fractional factorial experimental design. Linearity was established in the range of LOQ level to 0.2% having regression coefficients 0.9977. Calibration curve – y = 0.0132 + 0.9902. Since Δt for the content of quinabut is less than max δ, the technique is stable over time. The possibility of contamination of the sample by decomposition products by keeping it under stressful conditions (irradiation of the substance solution with UV light (UV irradiation with mercury lamp light); acid hydrolysis with 0.1 M hydrochloric acid solution; oxidative decomposition) was investigated. As a result of the irradiation with UV light, the impurity peaks for about 8.74 min (impurity C) and 12.68 min (impurity B) are additionally revealed. Their content exceeds the limits of normalization and is 0.6% and 3.7%, respectively. Therefore, the powder of the substance and its solutions should be stored away from direct sunlight. The column temperature and the speed of the mobile phase within ± 10% did not significantly affect the test results. The results were found to be within the assay variability limits during the entire process.

Conclusion. 1) The optimization of a new analytical method capable of simultaneous determination of quinabut assay and its impurities drug products was performed with a single fractional factorial experimental design. Only 11 experiments were needed for the optimization, while at least 16 experiments would be needed to cover the same analytical method operational region of the first optimization step with a traditional one factor at time (OFAT) approach. 2) HPLC method was developed and validated for the simultaneous detection and quantitation of quinabut and its impurities. 3) The final analytical method optimized with QbD approach was validated according to ICHQ2R1 guideline. The method proved to be sensitive, selective, precise, linear, accurate and stability-indicating. 4) The method was successfully applied to the analysis of demonstrating acceptable precision and adequate sensitivity for the detection and quantitation of quinabut and its impurities. So it may be reasonable to claim that the method can be extended to the analysis of drug formulations and stability samples as well. This optimization reflects in saving of time and resources since one stability study includes hundreds of samples tested during the product's shelf life.

Keywords

Quality by Design, HPLC, Quinabut, Impurities, Validation

Introduction

Nowadays non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most effective groups of drugs with characteristic anti-inflammatory, analgesic, antirheumatoid and antipyretic effects (Nasanov 2003; Simon et al. 2004; Balabanova 2010; Levin et al. 2012). However, the effectiveness of modern therapy for anti-inflammatory conditions cannot be called perfect. The main problems with the use of existing anti-inflammatory drugs are a large number of side effects, especially the course of inflammatory processes in various diseases and the need for long-term therapy of patients with concomitant diseases (Feuba 2004; Warner et al. 2004). There are no absolutely safe NSAIDs, and including selective COX inhibitors, and diclofenac sodium is still recognized as "gold" standard of effectiveness. Therefore, the problem of creating new drugs with high anti-inflammatory activity, selectivity and better safety parameters remains relevant over time.

Previously, a complex of pharmacological and biochemical studies found a high anti-inflammatory activity of the original active pharmaceutical ingredient Quinabut for the pharmacocorrection of inflammatory conditions, adjuvant arthritis in experimental animals (Kovalenko et al. 2012; Semenenko et al. 2013; Grib et al. 2015) (Fig. 1). It is shown that this substance is not inferior to the efficacy of the comparison drug diclofenac sodium and, importantly at therapeutic doses, does not show gastrotoxicity. In addition, the substance is characterized by antipyretic and antiproliferative action against the alternative phase of the inflammatory reaction. Considering the possibility

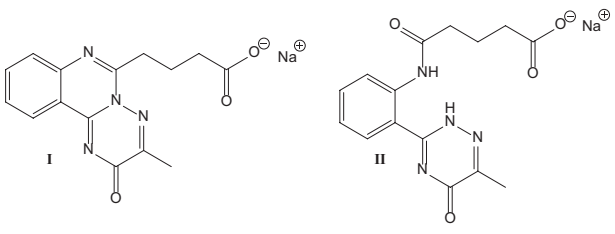

Figure 1. Chemical structures of quinabut (**I**) and impurity A (**II**).

of further use of Quinabut in medical practice, a necessary and mandatory prerequisite is the development of quality control of methods. That is, careful monitoring of the future drug from the initial stage of its introduction (preclinical studies), namely the development of methods for the quantification and determination of impurities, is a priority. All the more, so control of pharmaceutical impurities is currently a critical issue in the pharmaceutical industry. The International Conference on Harmonization (ICH) has formulated a workable guideline regarding the control of impurities. Organic impurities associated with the active pharmaceutical are the unwanted chemicals which are developed during drug synthesis or formulation. The presence of these unwanted chemicals, even in small amounts, may influence the efficacy and safety of the pharmaceutical products. Impurity profiling (identification and quantification) is now receiving increased attention from regulatory authorities.

Efforts for applying concepts of quality by design (QbD) principles to analytical method development have increased in recent years in order to achieve more accurate, robust and rigged analytical methods which are used for better control strategy of production processes. While quality by design principles are well known and adopted for the development of pharmaceutical products. QbD concept has not yet been fully adopted for analytical method development and optimization. So it was thought proper to use this combination for the present study for development of QbD based HPLC method. Therefore, it was thought desirable to develop a simple and accurate procedure that could be applied for the determination of quinabut and its impurities. The method was validated according to ICH Q1A (R2), Q2A and Q2B guideline (Q1A (R2) ICH Harmonized Tripartite Guideline 2003; Q2A ICH Harmonized Tripartite Guideline 1994; Q2B ICH Harmonized Tripartite Guideline 1996; Q8(R2) ICH Quality by design approach 2009). The results are reported in this paper.

The aim of study was to develop and validate a simple, highly robust (quality by design (QbD) approach), precise and accurate method using high performance liquid

chromatography (HPLC) for the simultaneous determination of original active pharmaceutical ingredient Quinabut and its impurities.

Materials and methods

Chemicals and reagents

"Quinabut" (sodium 4-(3-methyl-2-oxo-2*H*-[1,2,4]triazino[2,3-*c*]quinazolin-6-yl)butanoate **I**) – an original substance and impurity A (sodium 5-((2-(6-methyl-5-oxo-2,5-dihydro-1,2,4-triazin-3-yl)phenyl)amino)-5-oxopentanoate **II**) synthesized at the Department of Organic and Bioorganic Chemistry of Zaporizhzhya State Medical University (Head of the Department of Organic and Bioorganic Chemistry, Doctor of Pharmaceutical Sciences, Professor Kovalenko S.I.) [12, 13] (Fig. 1). Acetonitrile (HPLC grade) and orthophosphoric acid were procured from Sigma-Aldrich (USA).

Instrumentation and chromatographic conditions

Experiments were performed on a Shimadzu LC-20 Prominence HPLC separation module, equipped with a LC-20AD quaternary pump, a CTO-20A column oven, a SIL-20A autosampler, a diode array detector SPD-M20A (Shimadzu Corporation, Japan). Instrument control was performed using LC-20 Chemstation Software for chromatography (Shimadzu Corporation, Japan). Same software was used for data acquisition and processing of results. X-Terra RP18 (4.6×150 mm, 5 μm) analytical chromatographic column provided by Waters Corporation (Milford, MA) was used for all optimization experiments.

Mobile phase A: *acetonitrile R*. Mobile phase B: 0.025 M phosphate buffer solution. Samples were chromatographed in gradient mode (Table 1). Flow rate of the mobile phase: 0.7 mL/min. Column temperature: 40 °C. Detection: at 233 nm wavelength. Injection volume: 50 μl.

Chromatograph solvent (blank chromatogram), solution for checking the suitability of the chromatographic system.

Table 1. Gradient mode.

Chromatography time, min	Mobile phase A, %	Mobile phase B, %
0–10	7	93
10–40	45	55

The chromatographic system is considered suitable if:

- standard deviation of the base peak area for 5 injections not more than 2.0%;
- number of theoretical plates of the main peak is not less than 3000;
- the symmetry coefficient of the main peak is from 0.8 to 2.0;
- the separation coefficient calculated for the peaks of impurity A and sodium 4-(3-methyl-2-oxo-2H-[1,2,4]triazino[2,3-c]quinazolin-6-yl)butanoate, is not less than 1.5.

Standard solutions and sample preparation

Test solution (*a*). 25 mg (exact weighting) of the Quinabut substance is placed in a 50.0 mL volumetric flask, dissolved in 35 mL of *water R*, and the volume of the solution is brought up to the mark with the same solvent, mixed thoroughly. Filter through a membrane filter with a pore diameter of no more than 0.45 μm.

Test solution (*b*). 50 mg (exact weighting) of the Quinabut substance is placed in a 200.0 mL volumetric flask, dissolved in 150 mL of *water R*, and the volume of the solution is brought up to the mark with the same solvent, mixed thoroughly. Filter through a membrane filter with a pore diameter of no more than 0.45 μm.

Reference solution (*a*). 1.0 mL of the test solution is placed in a 50.0 mL volumetric flask, the volume of the solution is adjusted to *water R*, and mixed. 1.0 mL of the obtained test solution is placed in a 10.0 mL volumetric flask, the volume of the solution is adjusted to *water R*, and mixed.

Reference solution (*b*). 5.0 mg of sodium 4-(3-methyl-2-oxo-2H- [1,2,4]triazino[2,3-c]quinazolin-6-yl)butanoate is placed in a 100 mL volumetric flask, dissolved in 50 ml of *water R*, the volume of the solution is brought to the mark with the same solvent and mixed.

All solutions are used freshly prepared.

Method validation

The optimized analytical method was validated for determination of quinabut and its impurities. Linearity, precision, accuracy, limit of detection and quantification were determined for quinabut and its impurities. The acceptance criteria for different validation parameters were set in line with ICH requirements (Q1A (R2) 2003; Q2A 1994; Q2B 1996).

Specificity

A solution containing 0.002 mg/mL each was analyzed on HPLC using the above-given method.

A wavelength of 233 nm was chosen for analysis, since quinabut and its impurities have a maximum absorption or a shoulder in the UV spectrum (Fig. 2). This wavelength is the most convenient for taking chromatograms of quinabut and its impurities..

Limit of Detection (LOD) / Limit of Quantitation (LOQ)

The detection limits were determined on the basis of signal-to-noise (S/N) ratio ≥ 3 : 1 according to ICH guidelines. The quantitation limits were determined on the basis of signal-to-noise (S/N) ratio ≥ 10 : 1 according to ICH

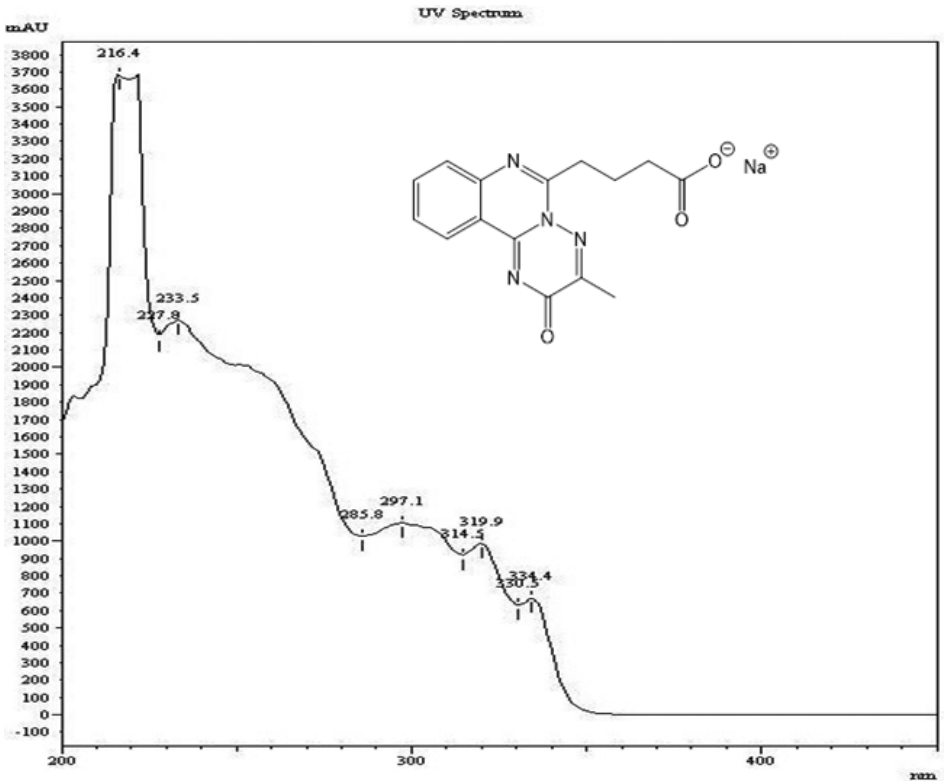

Figure 2. UV spectrum of quinabut.

guidelines. Six injections each of solutions containing concentrations equivalent to LODs and LOQs were performed to establish precision.

Linearity and range

The linearity of the method for determination of quinabut was determined by using nine different standard solutions of quinabut working standard. All solutions were prepared in three replicates. The covered concentration range was 80–120% of target concentration. Graphs of peak area against concentration were plotted for using a linear regression model.

Accuracy

The accuracy of the proposed analytical method was checked at three concentration levels. For quinabut determination the range was from 70%–130% of the target assay determination concentration. All samples were prepared by spiking the appropriate amount of a component into a placebo solution. Spiked samples were prepared in three replicates and analyzed by the proposed optimized analytical method.

Precision

The precision of the method was established by the study of repeatability (system precision), reproducibility (method precision) and intermediate precision. The repeatability was checked by making six injections of a solution containing 1 mg/mL each and % RSD was calculated for peak areas. 2 mg/mL each of six different solutions were prepared and analyzed. Intermediate precision was performed by a second analyst on a different day using a different instrument.

Robustness

The robustness of the method was established by minor changes in chromatographic conditions by varying column temperature and the flow of the mobile phase. The flow rates were changed from 0.6 mL/min to 0.8 mL/min, while temperature was changed from 35 °C to 45 °C. In all these experiments, concentration was 1 mg/mL.

Results and discussion

Screening of the influence of four chromatographic factors on different chromatographic responses was performed as the initial step of analytical method optimization. A randomized fractional factorial experimental design (2^{4-1}) of resolution IV with central point was used. Buffer pH, amount of acetonitrile in mobile phase A, the amount of phosphate buffer solution in mobile phase B and column temperature were selected as factors of interest, and were used to generate the fractional factorial experimental design. All factors and their corresponding levels are shown in Table 2. Fractional factorial experimental design was generated using Umetrics MODDE 11.0 software.

Six chromatographic responses presented in Table 3 were selected and measured for all performed experiments:

Table 2. Factors and corresponding levels for 2^{4-1} fractional factorial design used for screening analysis.

Factor name	Abbr.	Settings	Initial
(f1) Buffer pH	pH	2.6 to 3.4	3.0
(f2) Amount of PBS	PBS	45 to 65 mL	55 mL
(f3) Amount of ACN	ACN	35 to 55 mL	45 mL
(f4) Column temperature	Temp	30 to 50 °C	40 °C

Table 3. Responses and used suitability criteria for method optimization and sweet spot analysis.

Response name	Abbr.	Suitability criteria		
		Min	Target	Max
Resolution between quinabut peak and impurity A peak	Res 1	1.5	3.5	–
Resolution between quinabut peak and impurity B peak	Res 2	1.5	16.2	–
Resolution between impurity A peak and impurity B peak	Res 3	1.5	14.9	–
Number of theoretical plates of quinabut peak	N	31108	77754	–
Symmetry factor for quinabut peak	T	1.02	1.11	2.0
Retention time or quinabut peak	Rt	–	23.5	25.5

resolution between quinabut peak and impurity A peak (Res 2), resolution between quinabut peak and impurity B peak (Res 2), resolution between impurity A peak and impurity B peak (Res 3), number of theoretical plates of quinabut peak (N), symmetry factor for quinabut peak (T) and retention time or quinabut peak (Rt).

Eleven experiments presented in Table 4 were carried out according to the generated experimental design. Three central point experiments (experiment N9, N10 and N11) were also included for the determination of experimental error. All experiments were carried out in a randomized order (run order) in order to eliminate any systematic errors. The results of experiments are presented in Table 4. Abbreviation presents in Tables 2, 3. All obtained and

Table 4. Randomized 2^{4-1} fractional factorial design and results of observed responses.

Exp	Run	Factors				Responses					
No	order	f1	f2	f3	f4	Res 1	Res 2	Res 3	N	T	Rt
N1	1	–	–	–	–	2.44	1.53	2.48	77976	1.59	25.5
N2	11	+	–	–	+	1.98	1.51	1.98	83791	1.66	25.4
N3	3	–	+	–	+	1.55	1.57	2.51	69301	1.51	22.8
N4	4	+	+	–	–	1.78	1.72	1.43	74001	1.53	23.5
N5	8	–	–	+	+	1.51	1.59	1.67	63612	1.45	22.1
N6	2	+	–	+	+	1.65	1.61	1.81	65379	1.45	21.5
N7	7	–	+	+	–	1.71	1.83	2.62	56435	1.37	22.6
N8	10	+	+	+	–	1.62	1.67	1.71	61381	1.37	21.9
N9	9	0	0	0	0	2.53	2.52	2.71	75261	1.51	22.8
N10	5	0	0	0	0	2.52	2.51	2.67	71862	1.54	22.9
N11	6	0	0	0	0	2.53	2.61	2.68	72171	1.53	22.8

collected response measurements were processed with Umetrics MODDE software. Partial least squares (PLS) multivariate method of simultaneously estimating the models for all the responses was used for fitting and optimizing the statistical model. This provides an overview of the relationship between the responses and factors to determine the proper effect on all responses obtained within the statistical model.

The test requirements for checking the suitability of the chromatographic system are fulfilled, therefore the chromatographic system is considered suitable. Blank-solution (solvent), test solution, solutions for checking the chromatographic system were prepared to determine the specificity (Figs 3, 4).

Higher amount of ACN and PBS in the mobile phase have negative effect on theoretical plates of quinabut peak (N). In addition, higher level of these two factors also has a negative effect on symmetry factor of quinabut peak (T) and retention time of quinabut peak (Rt). Higher amount of ACN and PBS in the mobile phase with the combination of

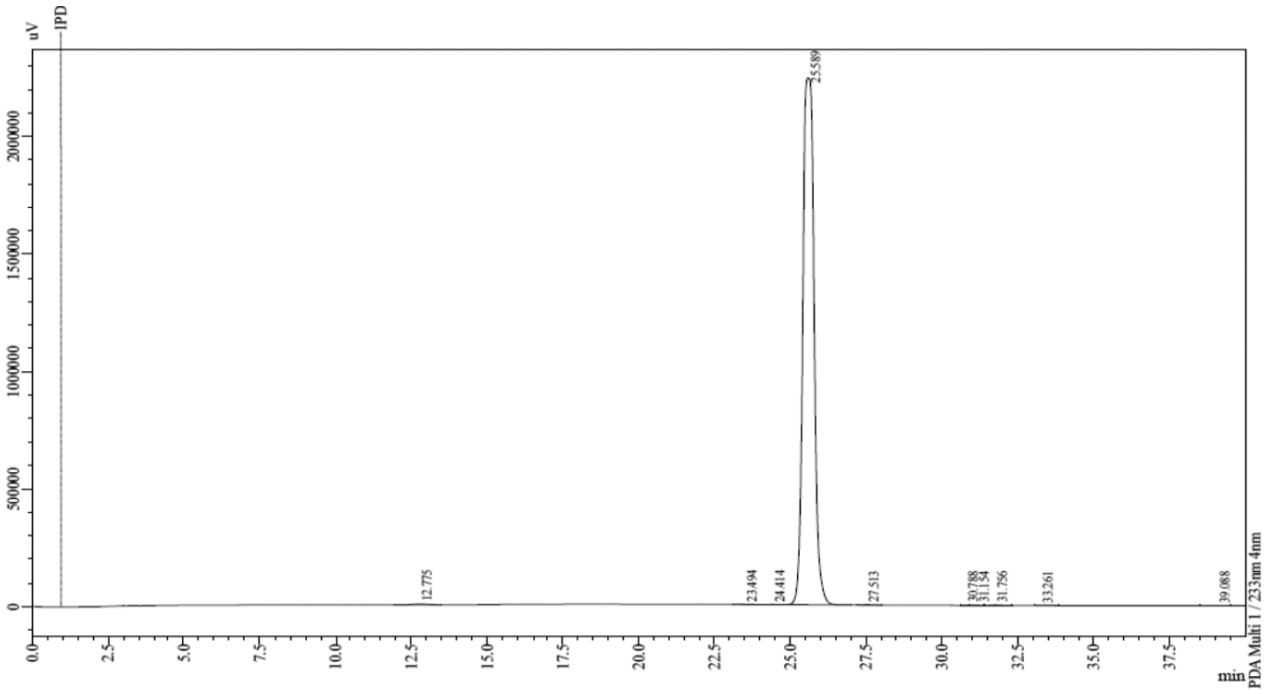

Figure 3. Chromatogram of the test solution.

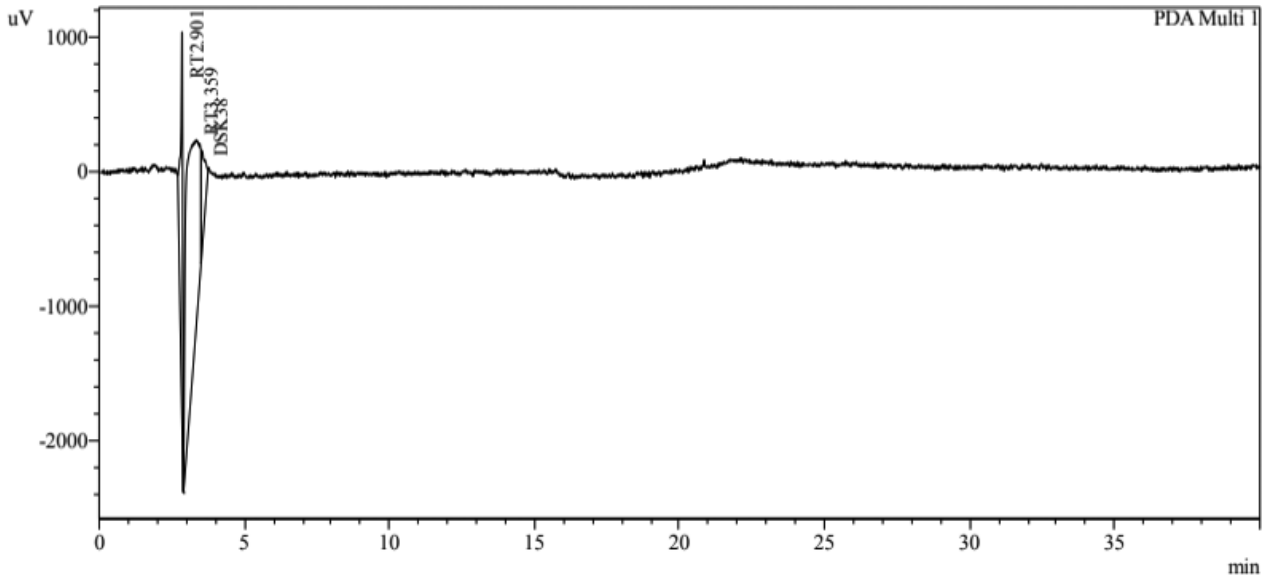

Figure 4. Chromatogram of blank solution.

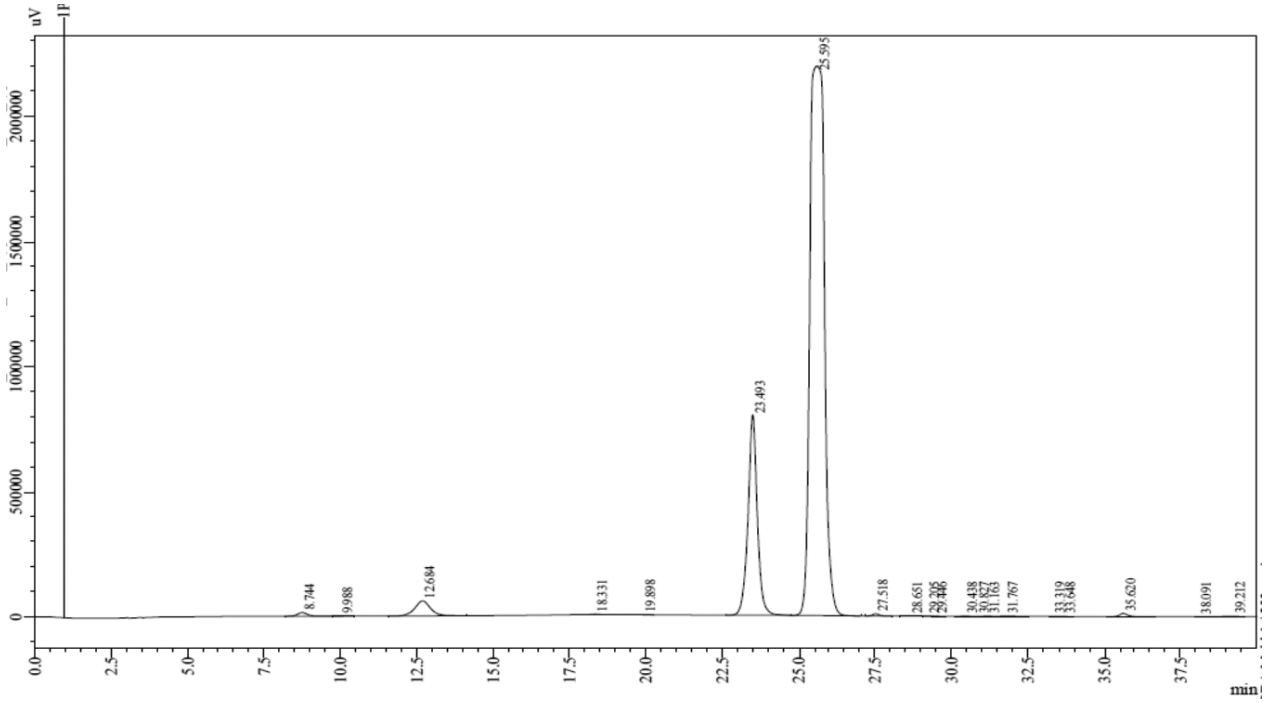

Figure 5. Chromatogram of the test solution (a) after exposure to UV light.

higher column temperature also have negative effect on the resolution between quinabut peak and impurity A (Res 1).

On the contrary, higher amount of ACN and PBS in the mobile phase have positive effect on resolution between impurity B peak and quinabut peak (Res 2), by improving the resolution between these two peaks. The most significant factors affecting the resolution between impurity A peak and impurity B peak (Res 3) are buffer solution pH with negative effect and the amount of PBS in the mobile phase with positive effect. Reducing the amount of ACN in the mobile phase and reducing the buffer solution pH would lead to better responses of all measured resolutions. All non-significant factors were excluded from the statistical model and the model was refitted. A representative chromatogram depicting resolution between all the components is shown in Fig. 5. The results indicated good resolution between the components with satisfactory peak shapes.

The possibility of contamination of the sample by decomposition products by keeping it under stressful conditions (irradiation of the substance solution with UV light (UV irradiation with mercury lamp light); acid hydrolysis with 0.1 M hydrochloric acid solution; oxidative decomposition) was investigated. As a result of the irradiation with UV light, the impurity peaks for 7.06 min (impurity C) and 12.68 min (impurity B) are additionally revealed. Their content exceeds the limits of normalization and is 0.6% and 3.7%, respectively. Therefore, the powder of the

substance and its solutions should be stored away from direct sunlight.

Comparison of chromatograms shows that in the conditions of the procedure for the determination of impurities, neither solvent, nor mobile phase, nor the main substance, interfere with the specificity of the method, do not interfere.

Limit of detection (LOD): LD ≤ 32% for limit tests. LOD ≤ max LOD = 0.32 * ImpLim (or 32% of ImpLim). The maximum content of a single impurity in a substance according to the method of determination should be no more than 0.2%. LOD = 0.32 * 0.2% = 0.064% of the substance content. Under the conditions of the method, the concentration of the test solution relative to the substance is about 0.5 mg/mL. Thus, the estimated LODimp ≤ 0.064% * 0.5 = 0.00032 mg/mL ≈ 0.32 μg /mL.

Minimum acceptable ranges of application of the method for quantitative determination of medicinal substances are from 80% to 120% of the nominal content. The following critical values for the linearity, precision, and correctness parameters for the quantitative content test for substances are also set: range = 80–120%; step = 5; Sy = 13.69; B = 2.0%; maxΔas = 2.0%; max δ = 0.64%; max S_0 = 1.06%; min r = 0.9970; max a = 3.2%.

The detection limits were determined on the basis of signal-to-noise (S/N) ratio ≥ 3 : 1 according to ICH guidelines. The LOD was 0.084 % (3.3 × Sa / b), LOQ was 0.25 % (10 × Sa / b). Table 5 and Fig. 6 summarize the results obtained for linearity for quinabut. Linearity was established in the range of LOQ level to 0.2% having regression coefficients 0.9977. Calibration curve – y=0.0132+ 0.9902.

The results obtained from the linearity study were used to evaluate accuracy and precision. To find accuracy and precision, the ratio found: entered: in percent (Zi): Zi = Yi / Xi * 100% in the normalized coordinates. The accuracy was evaluated based on the analysis of the sample with known concentration and comparison of the measured value with the true value of sodium 4-(3-methyl-2-oxo-2H- [1,2,4]triazino[2,3-c]quinazolin-6-yl)butanoate, which are part of the model mixture. To evaluate the accuracy, Δ_{AS} was used, which should not exceed the maximum allowable uncertainty of the test:

$$\Delta_Z = SD_z * t(95\%, g-1) \le \max \Delta_{AS}$$

To evaluate precision, the degree of correspondence between the known true value and the value obtained by this method was determined. The criterion for statistical insignificance.

If g = 9, then the methodology has a significant systematic error, since the value of δ% = 0.36% is greater than 0.33% (does not satisfy criterion 1) and is slightly different from zero, i.e. the criterion of practical insignificance δ% = 0, 36% ≤ 0.64% (satisfies criterion 2). The results of accuracy and intra-laboratory precision are given in Tables 6–9.

Thus, the technique is characterized by high intra-laboratory precision over the entire concentration range close to the nominal concentration. As presented in Tables 6–9 accuracy and precision for the determination of quinabut are acceptable.

The method for quantitative determination of quinabut does not specify the time after which the peak area is measured, so its stability was checked over time. Peak area

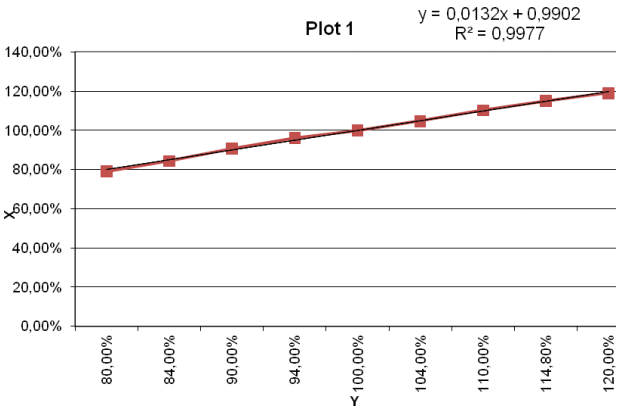

Figure 6. Linear dependence of the theoretical concentration of quinabut on the found concentration in normalized coordinates.

Table 5. Metrological characteristics of linear data.

Index	Value	Critical values for tolerances (80–120) %, the number of points g = 9	Conclusion
b	0.990214	–	–
s_b	0.025127	–	–
a	0.01323	1) ≤ 1.8946*s_a, 2) if not executed 1), than ≤ 3.2	performed
s_a	0.025251	–	–
s_r	0.009815	≤ max S_0 = 1.06 %	performed
r	0.997754	≥ min r = 0.9970	performed

Table 6. Results of recovery experiments.

Level	Concentration range	Mean recovery	% RSD
1	80 % (415 μg/mL)	101.31	0.16
2	100 % (500 μg/mL)	101.18	0.29
3	120 % (625 μg/mL)	101.17	0.25

Table 7. Intra-laboratory precision (first analyst).

Solution number	Weight, mg	Added in % to the concentration of the reference solution	Mean peak area	Found in % to the concentration of the reference solution	Found in % to added
MP	m_0	$X = \frac{C_i}{C_{st}} \times 100\%$	A	$Y = \frac{A_i}{A_{st}} \times 100\%$	$Z = \frac{Y_i}{X_i} \times 100\%$
AC1	25.1	100.40%	57554213	100.58%	100.18%
AC2	25.2	100.80%	57441256	100.38%	99.59%
AC3	25	100.00%	57895562	101.18%	101.18%
AC4	25.1	100.40%	57421002	100.35%	99.95%
AC5	25	100.00%	57892312	101.17%	101.17%
AC6	25	100.00%	57888963	101.16%	101.16%

Table 8. Intra-laboratory precision (second analyst).

Solution number	Weight, mg	Added in % to the concentration of the reference solution	Mean peak area	Found in % to the concentration of the reference solution	Found in % to added
MP	m_0	$X = \frac{C_i}{C_{st}} \times 100\%$	A	$Y = \frac{A_i}{A_{st}} \times 100\%$	$Z = \frac{Y_i}{X_i} \times 100\%$
AC1	25.1	100.40%	57215489	99.99%	99.59%
AC2	25.2	100.80%	57666458	100.78%	99.98%
AC3	25	100.00%	57667821	100.78%	100.78%
AC4	25.1	100,40%	56425133	98.61%	98.21%
AC5	25	100.00%	56998731	99.61%	99.61%
AC6	25	100.00%	57004235	99.62%	99.62%

Table 9. The results of the in-laboratory precision test.

№	Value Zi	
	Experiment 1	Experiment 2
1	100.18%	99.59%
2	99.59%	99.98%
3	101.18%	100.78%
4	99.95%	98.21%
5	101.17%	99.61%
6	101.16%	99.62%
$\bar{z}$	100.54%	99.63%
Z_{intra}, %	100.08%	
S_z	0.72%	0.83%
$RSD_{intra}\bar{z}$	0.88%	
Δ_{intra}	0.69 % < 2.0%.	

measurements of the test solutions were performed with a time interval of 24 hours for quinabut model solutions and reference solution (b). The stability results are listed in Table 10.

Table 10. Stability data of quinabut.

	t, hour			Average	RSD_t, %	Δ_t, %	max δ, %
	0	24	48				
Test solution (b)	57554213	57421002	57667821	57547678.67	0.0021	0.005	0.64
Reference solution (б)	229222651	234001232	229781146	231001676.3	0.0113	0.024	

Since Δt for the content of quinabut is less than max δ, the technique is stable over time.

To test the robustness of the HPLC method, the stability of the solutions over time, the influence of the pH of the mobile phase, the influence of the subjective factor (various analysts), the composition of the mobile phase, the column temperature, and the velocity of the mobile phase should be investigated. Robustness data results are given in Table 11.

The column temperature and the speed of the mobile phase within ± 10% did not significantly affect the test results. The results were found to be within the assay variability limits during the entire process.

Table 11. Robustness data of the technique.

Parameter	Settings	Peak area
Column temperature	35 °C	57657899
	45 °C	58012546
	RSD, %	0.004
The speed of the mobile phase	0.6 mL/min	57894215
	0.8 mL/min	57547162
	RSD, %	0.004

Conclusion

The optimization of a new analytical method capable of simultaneous determination of quinabut assay and its impurities drug products was performed with a single fractional factorial experimental design. Only 11 experiments were needed for the optimization, while at least 16 experiments would be needed to cover the same analytical method operational region of the first optimization step with a traditional one factor at time (OFAT) approach.

HPLC method was developed and validated for the simultaneous detection and quantitation of quinabut and its impurities.

The final analytical method optimized with QbD approach was validated according to ICHQ2R1 guideline. The method proved to be sensitive, selective, precise, linear, accurate and stability-indicating.

The method was successfully applied to the analysis of demonstrating acceptable precision and adequate sensitivity for the detection and quantitation of quinabut and its impurities. So it may be reasonable to claim that the method can be extended to the analysis of drug formulations and stability samples as well. This optimization reflects in saving of time and resources since one stability study includes hundreds of samples tested during the product's shelf life.

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Supplementary material 1

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