Validation of the analytical method for Repaglinide residual amount determination on the surfaces of cleanrooms and pharmaceutical equipment by means of UV spectrophotometry

Andrey Bursakov
CEO
Pharmasyntez – Tyumen, LLC
Tyumen, Russia
a.bursakov@pharmasyntez.com

Nasima Abdrazakova
Quality service
Pharmasyntez – Tyumen, LLC
Tyumen, Russia
n.abdrazakova@pharmasyntez.com

Elena Kryazheva
QA department
Pharmasyntez – Tyumen, LLC
Tyumen, Russia
el.kryazheva@pharmasyntez.com

Olga Knysh
Department of Pharmaceutical Disciplines
Federal State Budgetary Educational Institution of Higher Education «Tyumen State Medical University» of the Ministry of Healthcare of the Russian Federation
Tyumen, Russia
knysho@mail.ru@pharmasyntez.com

Ekaterina Vaseva
Department of Pharmaceutical Disciplines
Federal State Budgetary Educational Institution of Higher Education «Tyumen State Medical University» of the Ministry of Healthcare of the Russian Federation
Tyumen, Russia
knysho@mail.ru@pharmasyntez.com

Ivan Glukharev
QA department
Pharmasyntez – Tyumen, LLC
Tyumen, Russia
i.gluharev@pharmasyntez.com

Abstract—**During the drugs production, one of the most important requirements of good manufacturing practice (GMP) is the equipment cleaning from active pharmaceutical substance residues, which has to be carried out to prevent cross-contamination in case of switching from the production of one drug to another. The use of a sufficiently sensitive, rapid and simple UV spectrophotomery technique for determining trace amounts of repaglinide, carried out to control the quality of cleaning and cleaning validation in pharmaceutical production, is described. This technique is validated for specificity, linearity, detection limit and quantification limit. The calibration plot is linear in the concentration range of 0.1420 - 0.2130 mg/ml. The detection limit is $9.87 \cdot 10^{-7}$, the limit of quantification is $2.96 \cdot 10^{-6}$.**

Keywords—validation, UV spectrophotometry, validation of analytical methods, pharmaceutical equipment cleaning, residual amount determination, repaglinide

I. INTRODUCTION

One of the main actions ensuring the cross-contamination prevention is a well-conducted cleaning of pharmaceutical cleanrooms and equipment after switching a drug production. The cleaning and disinfection sufficiency is confirmed, in particular, by monitoring the presence of residues of active pharmaceutical ingredients (API). In this regard, the development and confirmation of the effectiveness of the methodology for determining the residual amounts of API is one of the priority tasks of enterprises manufacturing drugs.

II. LITERATURE REVIEW

Repaglinide (lat. Repaglinidum, $C_{27}H_{36}N_2O_4$) is a derivative of carbamoyl-methyl-benzoic acid, an antidiabetic drug belonging to the meglitinide group. The chemical name of IUPAC is (S)-2-Ethoxy-4-[2-[[methyl-1-[2-(1-piperidinyl)-phenyl]butyl]amino]-2-oxoethyl]-benzoic acid (Fig. 1). The substance stimulates insulin secretion by blocking ATP-dependent potassium channels in the membranes of functionally active beta cells of the islet pancreatic apparatus (which leads to depolarization and opening of calcium channels). It has a pronounced sugar-lowering effect and a relatively highly safe influence on body weight. This drug may be recommended as monotherapy or combination therapy for diabetes mellitus [1].

Repaglinide is practically insoluble in water. Freely soluble in methanol and methylene chloride (table I) [2].
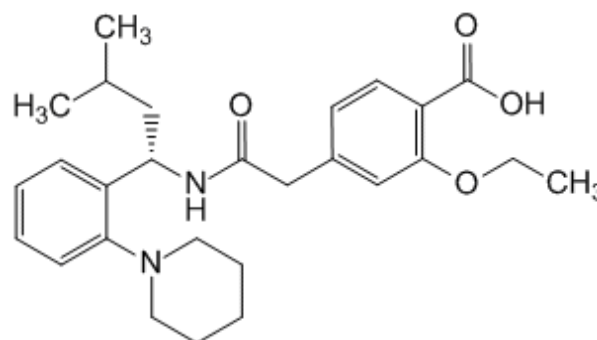

Fig. 1. Skeletal structural formula of repaglinide.

TABLE I. THE MAIN PHYSICO-CHEMICAL PROPERTIES OF REPAGLINIDE

1	Description	White or almost white crystal powder
2	Solubility	Freely soluble in methanol and methylene chloride, practically insoluble in water
3	Specific rotation	+6.3 – 7.7 (5% solution of repaglinide in methanol)

Cleaning validation, in particular validation of analytical methods, is a direct requirement of Good Manufacturing Practice (GMP) [3] and should be carried out for the successful validation of technological processes and the organization of disinfection and quality control at the enterprise.

III. EXPERIMENTAL

To determine the residual amounts of Repaglinide, a UV spectrophotometry technique was used. This method is based on determining the degree of absorption of monochromatic light by a solution depending on the concentration of substance. The advantages of this method are high sensitivity, simplicity and rapidity. In case of determination of trace amounts of non-toxic substances, the UV spectrophotometry technique can be applied [4–7] along with the more expensive and time-consuming high performance liquid chromatography (HPLC) method, which is often used to control the quality of cleaning [8–12].

Equipment: laboratory electronic scales OHAUS Pioneer PA-214C, spectrophotometer SHIMADZU UV-1800, ultrasonic bath SONOREX RK 102 H, orbital shaker LOIP LS-221.

Materials: graduated glass volumetric flask 25 ml, graduated glass volumetric flask 100 ml, chemical glass 100 ml, graduated volumetric pipette 10 ml, graduated volumetric pipette 1 ml, probe swab, repaglinide standard solution, methanol solvent, test solution, methanol, purified water, placebo powder.

IV. RESULTS AND DISCUSSION

A. Validated method

Reference and test solutions were prepared for analyses.

To prepare a standard solution, 0.1775 g of repaglinide was placed in a 100 ml volumetric flask, 50 ml of solvent was added, shaken on an orbital shaker at 250 rpm for 3 minutes, the volume of the solution was adjusted to the mark with solvent. 10 ml of the prepared solution was transferred into a volumetric flask with a capacity of 100 ml, brought to the mark with a solvent. Transfer 0.4 ml of this solution into a 10 ml volumetric flask, bring the solvent to the mark.

To prepare the test solution, a probe swab was moistened with a solvent. After wetting the head of the swab with a solvent, the excess liquid was squeezed out, pressing the swab to the wall of the tube. A swabbing was made from a surface area of 25 cm^2. After taking the swabs, probe samples were placed in a test tube with 5 ml of solvent. The contents of the probe swab were recovered by sonication for 5 minutes.

The optical density of the reference and test solutions was measured at a wavelength of 245 nm in a cuvette with a layer thickness of 1 cm relative to the solvent.

The optical density of the test solution did not exceed the optical density of the repaglinide reference solution.

B. Validation of analytical method

At a laboratory conditions, 9 solutions with a repaglinide content of 80%, 85%, 90%, 95%, 100%, 105%, 110%, 115%, 120% of the maximum allowable in swabs were prepared from a pharmaceutical substance (Table II).

TABLE II. REPAGLINIDE SOLUTIONS PREPARATION

№	Sample name	Test substance, g	Placebo, g	Solvent, ml
1	A0	0.0000		
2	A80	0.1420		
3	A85	0.1509		
4	A90	0.1598		
5	A95	0.1686		
6	A100	0.1775	0.0950	100
7	A105	0.1864		
8	A110	0.1953		
9	A115	0.2041		
10	A120	0.2130		

Then a sequential dilution was carried out: 10 ml of the resulting solution was transferred into a 100 ml volumetric flask, the volume of the solution was adjusted to the mark with the solvent and mixed. 1 ml of the resulting solution was transferred into a 25 ml volumetric flask, the volume of the solvent was adjusted to the mark and mixed.

C. Specificity

Specificity was valued by the model solutions absorption spectrums comparison.

During the validation, it was found that excipients do not affect the determination of the API, there is no peak at the placebo solution at a wavelength of 245 nm (Fig. 2-4).
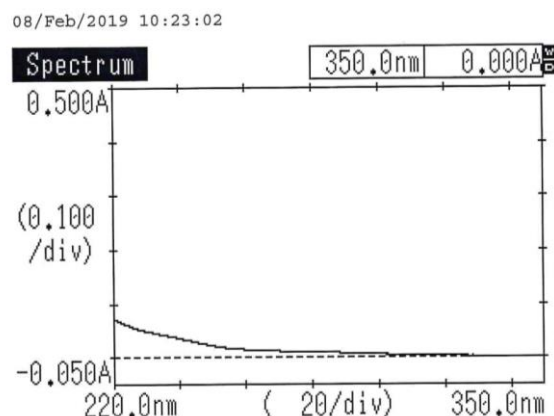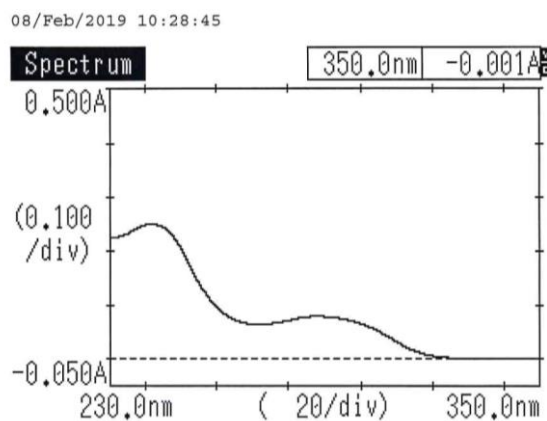

Fig. 2. UV spectra of placebo solution.

Fig. 3. UV spectra of solution A100.

Fig. 4. Peak detection of solution A100.

D. Lineary

Linearity was valued by measuring the repaglinide content in model solutions. The experimental data were processed using the least squares method using a linear model and calculating the correlation coefficient (r) (Table III).

The correlation coefficient is 0.993, the method is able to give results proportional to the amount of repaglinide in the sample (Fig. 5).

E. Determination of the detection limit (LOD), the limit of quantitation (LOQ) and the LOQ repeatability

It is generally accepted that the detection limit is a concentration that the signal exceeds the background by an amount equal to three times the standard deviation of the background signal.

TABLE III.   LINEARY DETERMINING

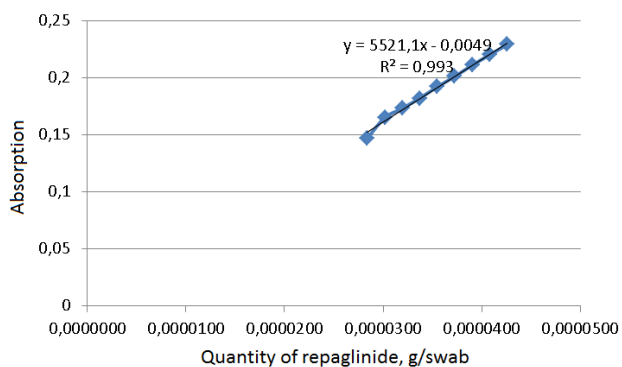| №	Model solution	Optical density	Conc., g/swab	Acceptance criteria	r	Result Pass/Fail
1	A80	0.147	$2.84 \cdot 10^{-5}$			
2	A85	0.165	$3.02 \cdot 10^{-5}$			
3	A90	0.173	$3.20 \cdot 10^{-5}$			
4	A95	0.182	$3.37 \cdot 10^{-5}$			
5	A100	0.192	$3.55 \cdot 10^{-5}$	r≥0,99	0.993	Pass
6	A105	0.201	$3.73 \cdot 10^{-5}$			
7	A110	0.212	$3.91 \cdot 10^{-5}$			
8	A115	0.220	$4.08 \cdot 10^{-5}$			
9	A120	0.229	$4.26 \cdot 10^{-5}$			

Fig. 5. Linearity chart

A number of background optical density values (comparison solution) were obtained. The standard deviation of the background signal was calculated. The detection limit was found by the following formula:

$$LOD = \frac{3 \cdot \sigma}{S}, \qquad (1)$$

here σ is the standard deviation of the background signal,

S is the slope of the calibration graph.

The LOQ value is three times greater than the LOD value. Thus, the following formula will be used to calculate the LOQ:

$$LOQ = 3 \cdot LOD, \qquad (2)$$

Repeatability of LOQ:

Six solutions were prepared as follows. To prepare the initial solution, a sample of 0.0261 g of repaglinide was taken, transferred to a volumetric flask with a capacity of 100 ml, brought to the mark with a solvent. Then, sequentially in 6 volumetric flasks with a capacity of 100 ml, 1 ml of the initial solution was added, the solutions were brought to the mark with a solvent. Photometrics performed.

The results of determining the detection limit and the limit of quantification are given in Tables IV-VI.

Formula for calculation:

$$X = \frac{A + 0.0049}{5521.1}, \qquad (3)$$

A – optical density;
0.0049 – variable;
5521.1 – angular coefficient;
X – concentration, g/ml.

TABLE IV.   DETERMINING OF LOD AND LOQ

№	Optical density	Mean value	Standard deviation	LOD, g/swab	LOQ, g/swab
1	0.004				
2	0.003				
3	0.000	0.0016	0.00182	$9.87 \cdot 10^{-7}$	$2.96 \cdot 10^{-6}$
4	0.000				
5	0.001				

TABLE V.   DETERMININD OF THE LOQ REPEATABILITY

Solution number	Weight, g	Dilution			Conc., g/swab	Optical density
		Dilution to, ml	Aliquot volume, ml	Dilution to, ml		
1			1	100	$2.90 \cdot 10^{-6}$	0.014
2			1	100	$2.90 \cdot 10^{-6}$	0.014
3			1	100	$2.90 \cdot 10^{-6}$	0.015
4	0.0261	100	1	100	$2.90 \cdot 10^{-6}$	0.014
5			1	100	$2.90 \cdot 10^{-6}$	0.016
6			1	100	$2.90 \cdot 10^{-6}$	0.014

TABLE VI.   STATISTICAL PROCESSING

Parameter	Acceptance criteria	Actual value	Result Pass/Fail
Relative error, %	≤5	1.35%	Pass

The ability of the method to reproduce the effectiveness at the lowest concentration of the substance in the sample is shown.

V.   CONCLUSION

It was found that the proposed method is characterized by a satisfactory linearity, and its sensitivity allows to quantify the content of repaglinide residues on the pharmaceutical equipment.

The successful validation of the UV spectrophotometry method for determining trace residues of repaglinide allows us to consider this method for monitoring the pharmaceutical equipment and cleanrooms cleaning from API that are not highly toxic.

ACKNOWLEDGMENT

REFERENCES

[1] T.Yu. Demidova, "Repaglinide: a possibility of clinical use for patients with diabetes type 2", Effective Pharmacotherapy, vol. 51, pp. 18-25, 2012 (in Russ.).

[2] European Pharmacopeia. 8th edition. EP 8.0. Reparlinide. 2008. (monograph 2135).

[3] Order of the Ministry of Industry and Trade of the Russian Federation dated June 14, 2013, № 916 as amended by Order of the Ministry of Industry and Trade of Russia dated December 18, 2015 No. 4148 "On Approval of the Rules of Good Manufacturing Practice".

[4] A.V. Egorova, A.A. Fedosenko, G.V. Maltsev, V.P. Antonovich, "Validation of quality control methods for cleaning pharmaceutical equipment". Analytics and control, vol. 19 (4), pp. 387-395, 2015 (in Russ.).

[5] S.K. Jain, G.P. Agarwal, N.K. Jain, "Spectrophotometric determination of repaglinide in tablet dosage forms". Indian journal of pharmaceutical sciences, vol. 67 (2), pp. 249-251, 2005.

[6] N. Patel, K.R. Patel, "Development and Validation of UV Spectrophotometric Method for Simultaneous Estimation of Metformin HCL and Repaglinide in Bilayer Tablet", Journal of Pharmaceutical Science and Bioscientific Research, vol. 5 (1), pp. 104-109, 2015.

[7] R.N. El-Shaheny, "Validated Stability-Indicating Spectrofluorimetric Method with Enhanced Sensitivity for Determination of Repaglinide in Tablets", J. og Fluoresc, vol. 22, pp. 1587-1594, 2012, https://doi.org/10.1007/s10895-012-1099-0.

[8] G.A. Shabir, "Equipment Cleaning Validation: Developing an HPLC Method to Determine Contamination Residues on Equipment Surfaces". Am. Pharm. Rev., vol. 11 (4) pp. 16-22, 2008.

[9] A.H. Schmidt, M. Stanic, "UPLC-MS/MS in support of cleaning validation studies. Cephalosporin antibiotics production facility", G.I.T. Labpratory J. Europe, vol. 5-6, pp. 28-30, 2012.

[10] M. Gandhimathi, T.K. Ravi, S.K. Renu, "Determination of repaglinide in pharmaceutical formulations by HPLC with UV detection", Analytical Sciences. vol. 19 (12), pp. 1675-1677, 2003.

[11] N. Kaushal, S. Jain, A.K. Tiwary, "Development of spectrofluorimetric and HPLC methods for in vitro analysis of repaglinide", Indian journal of pharmaceutical sciences, vol. 72 (2), pp. 240-244, 2010.

[12] S.M. Dhole, N.D. Amnerkar, P.B. Khedekar, "Comparison of UV spectrophotometry and high performance liquid chromatography methods for the determination of repaglinide in tablets", Pharmaceutical methods, vol. 3 (2), pp. 68-72, 2012.