Validation of Sedative Druganalysis Results

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Abstract

Annotation. The article reveals validation studies of the method of quantitative analysis of high-performance liquid chromatography of a complex and multicomponent original sedative drug produced in the Republic of Uzbekistan. Criteria for repeatability, accuracy, precision, intermediate accuracy and correlation coefficients for drug validation were determined. The analytical method for determining the number of tablets covered with a film "Sedarem" from the proportion of valeric acids in the main active ingredients of the drug was determined on the basis of the 5th series of the "Accuracy" indicator. The accuracy of valeric acid is judged from at least 5 data obtained for each of the 3 levels lying within the analytical domain of the quantification method. Comparing the results of determining the amount of valeric acid, it turns out that there is no statistically significant difference between them, that the three time points for each series are mutually compatible.

The analysis procedure for determining the amount of phenolic compounds in the preparation, when comparing "Precision" with the results of determining the amount of phenolic compounds at different intervals and on different days, which characterizes repetition and "intermediate precision," it was found that there is no statistical reliable difference between them, and three time points for each series are mutually compatible. Study of drug linearity from flavanoid fraction was carried out in the range from 80 to 120% of nominal value of analytical parameters.

Keywords*:"Sedarem", tablet, high performance liquid chromatography, repeatability, accuracy, precision, intermediate precision and correlation coefficient.*

Introduction

Due to the high efficacy of sedatives and the absence of side effects, these drugs are widely used in everyday medical practice, especially in the treatment of elderly patients. According to the classification, drugs based on medicinal plant raw materials are widely used among sedatives. Progress, life growth at a rapid pace further increases the need for sedatives[1,3].

An effective technology for preparing dosage forms with optimal technological indicators of the proposed original sedative has been developed, standardization methods, quality and validation standards have been established.

The validation criteria of the Sedarem sedative tablet quantitative analysis method, coated with cladding, were determined by the following indicators: accuracy, repeatability, linearity, precision, intermediate accuracy and correlation coefficient[3].

Aim of the Research: Study the possibility of determining the validation criteria for Sedarem with a coating.

Materials and methods: Quantitative analysis of the sum of the main active ingredients in the coated

tablet, i.e., carboxylic acids with respect to valeric acid, flavonoids with respect to routine, as well as phenolic compounds with respect to rosmarinic acid, was carried out on an Agilent Technologies 1200liquid chromatograph with a UV detector.

A suitable chromatographic column S-18 is Zorbax Eclipse with a particle size of 5 μm, filled with a sorbent of 4x100 m.

The validity criteria of the method of quantitative analysis of sedative tablets "Sedarem" coated with the shell were determined on the following parameters: accuracy, repeatability, linearity, precision, intermediate precision and correlation coefficient.To study the possibility of determining the criteria for validation of the drug "Sedarem" coated with the shell. Quantitative analysis of the sum of carboxylic acids relative to valerian acid, flavonoids relative to routine, and phenolic compounds relative to rosemary acid was performed on Agilent Technologies 1200 liquid chromatograph, UV detector.A suitable chromatographic column S-18 is a ZorbaxEclipse filled with 4x100 mm sorbent with a particle size of 5 mm.

The results were processed in the method of variance statistics, according to the Student's criterion, at p = 0,05. The tables show the arithmetic mean (M), the corresponding standard error of error (m), the Student Criterion (t), the number of choices (n), and the confidence limit (lower confidence limit ÷ high confidence limit)[4].

Results and Discussion

Validation of the method of quantitative determination of the medicinal form of the tablet coated with a shell, "Sedarem" on valerian acids "Correctness". It is known that the accuracy of the method is characterized by the deviation of the average result of its definitions from the values obtained as correct [1]. The tested method is considered valid if the values obtained as true are at reliable intervals corresponding to the average results of the analyses obtained as a test for this method. After determining the quantity of the preparation by the fraction of valerian acids in different series, the following results were obtained, and the results are given in Table 1. If we compare the results of determining the quantity of valerian acids in five different series of the preparation under test, there seems to be no statistically significant difference between them, the results of exactly five series are mutually compatible. The analytical method for determining the amount of Sedarem tablets coated with valerian acid corresponds to the requirements of validation on the indicator "Accuracy".

The accuracy of the method is explained by the dispersion of the results obtained using it relative to the average result value. The criterion of such dispersion is the value of standard deviation of the result of a specific unit obtained to select a sufficiently large volume 2,3. For any method of quantification of accuracy, the quantification method is estimated from the results of at least 5 data obtained for each of the 3 levels (low, medium and high) lying in the analytical domain of the method.

Repetition (similarity). Repeatability of the analytical method has been evaluated based on independent results obtained in a short time in the same laboratory under the same conditions. Intermediate precision in the laboratory. Intermediate accuracy in the laboratory of the approved method was evaluated in one laboratory5. Five sets of Sedarem-coated tablets were used in the analysis. After analyzing the tablets on the indicator "Precision", which characterizes the similarity of the repeatability of the results of quantitative determination of the drug, the following results are given in Table 1

If we compare the results of testing valeric acids of five different series of drugs, it becomes clear that there are no statistically reliable differences between them, exactly five series are mutually compatible[6].

After checking for intermediate precision, the following results were obtained and are presented in Table 1.

Table-1.: The results on the accuracy of the quantitative determination precision analysis repeatability and intermediate precision of the results of quantitative determination of the drug on the presence of valeric acids (M+tm; p=0,05; n=5)

Shell-coated tablets "Sedarem"	Repeatability	Accuracy of the quantitative determination	Intermediate precision		
			Results of experiments on the first day	Results of experiments on the second day	Results of experiments on the third day
Series: Experimental 1	4,3231 (4,1223÷ 4,5239)	4,6356(4,3211÷4,9501)	4,4321 (4,2131÷4,6511)	4,2651 (4,1021÷4,4281)	4,4265 (4,2045÷4,6485)
Series: Experimental 2	4,4755 (4,2312÷ 4,7198)	4,4564(4,2351÷4,6777)	4,5312 (4,3101÷4,7523)	4,5612 (4,3001÷4,8223)	4,3945 (4,2012÷4,5878)
Series: Experimental3	4,2894 (4,1356÷ 4,4432)	4,3261(4,1223÷4,5299)	4,6531 (4,2864÷5,0198)	4,4764 (4,2456÷4,7072)	4,4621 (4,2501÷4,6741)
Series: Experimental 4	4,4751 (4,1884÷ 4,7618)	4,5621(4,2311÷4,8931)	4,3125 (4,1754÷4,4496)	4,3355 (4,2347÷4,4363)	4,4123 (4,2845÷4,5401)
Series: Experimental 5	4,3241 (4,0894÷ 4,5588)	4,2313(4,0256÷4,4370)	4,4642 (4,2475÷4,6809)	4,4659 (4,2759÷4,6559)	4,3489 (4,1611÷4,5367)

If the results of determining the amount of ivalerian acids in different time intervals are compared, it appears that there is no statistically significant difference between them, that the three time points for each series are mutually compatible. The method of analysis for quantitative determination of valeric acid content of cedarem shell-coated tablet meets the validation requirements on the indicators of "Precision", which characterizes the repeatability (similarity) and "Intermediate precision". The linearity of the method is that the method of the analytical signal depends on the concentration or amount of the analyte detected in the sample under analysis, within the scope of the analysis. In method validation, its linearity in the field of analysis is verified by testing, by analytical measurements for at least five samples with complete amounts or concentrations of the analyte.

To determine the linearity of the range in the analytical field (80–120%), we calculated a correlation coefficient (R), which should be $R \geq 0,99$.

Research results. Due to the requirements for the size of the analytical area of the methodology in the range of 80 to 120% of the nominal value of the analytical indicator to be determined, the following results obtained are given in Table 2.

Table 2. Results of the study of the preparation linearity by the fraction of valerian acid, (in the analysis the area is 80-120% (n = 5)

№	Amount of sample taken,%	Series: Experimental 1	Series: Experimental 1	Series: Experimental 1	Series: Experimental 1	Series: Experimental 1
1	80	3,4556	3,3965	3,4894	3,4854	3,5622
2	90	3,9864	3,9864	4,0310	4,0054	3,9864
3	100	4,5612	4,3985	4,4895	4,6120	4,4561
4	110	5,1350	5,1312	5,0011	4,9984	5,0010
5	120	5,7845	5,6923	5,4759	5,3785	5,3986
Linear Connection Free Member (a)		-1,22186	-1,21542	-0,44572	-0,28326	-0,20654
Linear connection angle coefficient (b)		0,058064	0,057364	0,049431	0,047792	0,046874
Correlationcoefficient (R)		0,99936	0,99713	0,99971	0,99509	0,99894
Regression equation Y=aX+b		Y=-1,22186 X+0,058064	Y=-1,21542 X+0,057364	Y=-0,44572 X+0,049431	Y=-0,28326 X+0,047792	Y=-0,20654 X+0,046874

In accordance with the requirements of the 12 edition of GPh, in the joint venture "Remedy group" shell-coated tablets "Sedarem" meet the linearity of the drug in five series, in the field of analysis (from 80 to 120%).

The methods of analysis for determining the amount of valerian acid in a cedar-coated tablet met the validation requirements for the "Analysis Area" and "Linearity" indicators.

The validation of the method for determining the amount of phenolic compounds in shell-coated tablets "Sedarem". After determination of the quantity of the pharmaceutical form by the fraction of phenolic compounds in different series, the following data were obtained. The obtained results are presented in Table 3.

If the results of determining the amount of phenolic compounds in five different series of the tested drug are compared, there is no statistically significant difference between them, the results of exactly five series are mutually compatible. The method of analysis for the determination of the amount of phenolic compounds in the drug "Sedarem" coated tablets meets the requirements

of validation on the indicator "Accuracy". Following the determination of the drug on the indicator "Precision", which characterizes the repeatability of the results of the determination of the amount of the drug, the following data were obtained (Table 3).

If we compare the study of five different series by the percentage of phenolic compounds in the tablet, there is no statistically significant difference between them, the results of exactly five series are mutually compatible.

After switching to intermediate precision, the following data were obtained (Table 3).

Table 3: The results on the accuracy of the quantitative determination precision analysis repeatability and intermediate precision of the results of quantitative determination of the drug on the presence of of phenolic compounds (M+tm; p=0,05; n=5)

Shell-coated tablets "Sedarem"	Repeatability	Accuracy of the quantitative determination	Intermediate precision		
			Results of experiments on the first day	Results of experiments on the second day	Results of experiments on the third day
Series: Experimental 1	24,2311 (22,3146÷26,1476)	23,8954 (21,5612÷26,2296)	23,5896 (21,5613÷25,6179)	24,2315 (22,0564÷24,2315)	23,7555 (21,8654÷25,6456)
Series: Experimental 2	24,6531 (21,8956÷27,4106)	24,8964 (22,3456÷27,4472)	24,0121 (22,8952÷25,1290)	24,0302 (22,1333÷25,9271)	24,0121 (22,1323÷25,8919)
Series: Experimental3	22,8964 (21,5654÷24,2274)	25,0012 (22,8031÷27,1993)	22,8961 (21,3564÷24,4358)	22,7451 (21,1111÷24,3791)	22,8995 (21,2082÷24,5908)
Series: Experimental 4	23,4796 (22,3131÷24,6461)	24,3561 (21,9864÷26,7258)	23,4656 (22,0121÷24,9191)	23,4512 (22,0101÷24,8923)	23,4122 (21,5894÷25,2350)
Series: Experimental 5	23,8564 (21,8896÷25,8232)	24,6551 (22,3123÷26,9979)	22,4512 (21,2898÷23,6126)	24,0089 (23,0012÷25,0166)	23,7451 (21,4955÷25,9947)

If we compare the results of determining the number of phenolic compounds at different time intervals and on different days, it turns out that there is no statistically significant difference between them, that three time points for each series are mutually compatible.

The method of analysis for determining the number of phenolic compounds in the form of shell-coated tablets "Sedarem"meets the requirements of the validation for "Accuracy", which characterizes the reproducibility and "Intermediate Accuracy".

In connection with requirements to the size of the analysis methodology area in the range from 80 to 120% of the nominal value of the identified analytical indicators, the following results were obtained (Table 4).

Table-4: Results of the study of linearity of the preparation in terms of the share of phenolic compounds, when the analysis area is 80-120% (n=5)

№	Amountofsampletaken,%	Series: Experimental 1	Series: Experimental 2	Series: Experimental3	Series: Experimental4	Series: Experimental 5
1	80	17,5641	16,5651	15,8955	16,0011	15,3256
2	90	20,2313	20,201	19,5621	20,1321	18,9655
3	100	24,3563	24,0203	23,4565	23,9986	22,3565
4	110	30,1232	29,8564	28,9564	28,9999	27,1635
5	120	34,2561	34,9856	33,8999	33,2561	32,5644
Linear Connection Free Member (a)		-17,9697	-21,37072	-21,04902	-18,90024	-19,4005
Linear connection angle coefficient (b)		0,432759	0,464964	0,454031	0,433778	0,426756
Correlationcoefficient (R)		0,99308	0,99503	0,99644	0,99917	0,99482
Regression equation Y=aX+b		Y=-17,9697 X+0,432759	Y=-21,37072 X+0,464964	Y=-21,04902 X+0,454031	Y=-18,90024 X+0,433778	Y=-19,4005 X+0,426756

In accordance with the requirements of the 12 edition of GPh, in the joint venture "Remedy group" shell-coated tablets "Sedarem" meet the linearity of the drug in five series, in the field of analysis (from 80 to 120%). The method of analysis for determining the amount of phenolic compounds in shell-coated tablets "Sedarem" meets the requirements of validation on the indicators "Area of analysis" and "Linearity".

Validation of the method of quantitative determination of cedarem shell-coated tablets by flavonoids

After determining the amount of flavonoids in different series of the drug, the following data were obtained (Table 5).

If the results of determining the amount of flavonoids in five different series of the tested drug are compared, there is no statistically significant difference between them, the results of exactly five series are consistent. The analytical method for determining the amount of flavonoids in the shell-coated tablets "Sedarem" met the validation requirements on the indicator "Accuracy".

After checking the dosage form on the indicator "Precision", which characterizes the repeatability of

the results of quantitative determination of the drug, the following results were obtained (Table 5).

Determining the proportion of flavonoids of the drug in five different series, there is no statistically significant difference between them, the results of exactly five series are mutually compatible.

The following data were obtained after the intermediate precision test analyzes (Table 5)

Table 5: The results on the accuracy of the quantitative determination precision analysis repeatability and intermediate precision of the results of quantitative determination of the drug on the presence of of flavonoids (M+tm; p=0,05; n=5)

Shell-coated tablets "Sedarem"	Repeatability	Accuracy of the quantitative determination	Intermediate precision		
			Results of experiments on the first day	Results of experiments on the second day	Results of experiments on the third day
Series: Experimental 1	4,3231 (4,1223÷ 4,5239)	4,6356(4,3211÷4,9501)	4,4321 (4,2131÷4,6511)	4,2651 (4,1021÷4,4281)	4,4265 (4,2045÷4,6485)
Series: Experimental 2	4,4755 (4,2312÷ 4,7198)	4,4564(4,2351÷4,6777)	4,5312 (4,3101÷4,7523)	4,5612 (4,3001÷4,8223)	4,3945 (4,2012÷4,5878)
Series: Experimental3	4,2894 (4,1356÷ 4,4432)	4,3261(4,1223÷4,5299)	4,6531 (4,2864÷5,0198)	4,4764 (4,2456÷4,7072)	4,4621 (4,2501÷4,6741)
Series: Experimental 4	4,4751 (4,1884÷ 4,7618)	4,5621(4,2311÷4,8931)	4,3125 (4,1754÷4,4496)	4,3355 (4,2347÷4,4363)	4,4123 (4,2845÷4,5401)
Series: Experimental 5	4,3241 (4,0894÷ 4,5588)	4,2313(4,0256÷4,4370)	4,4642 (4,2475÷4,6809)	4,4659 (4,2759÷4,6559)	4,3489 (4,1611÷4,5367)

If we compare the results of determining the number of phenolic compounds at different time intervals and on different days, it turns out that there is no statistically significant difference between them, that three time points for each series are mutually compatible.

The method of analysis for determining the number of phenolic compounds in the form of shell-coated tablets "Sedarem"meets the requirements of the validation for "Accuracy", which characterizes the reproducibility and "Intermediate Accuracy".

In connection with requirements to the size of the analysis methodology area in the range from 80 to 120% of the nominal value of the identified analytical indicators, the following results were obtained (Table 6).

Table-6: The results of the study of the linearity of the drug on the proportion of flavanoids, when the analytical area is 80-120% (n=5)

№	Amoun of sample taken,%	Series: Experimental 1	Series: Experimental 2	Series: Experimental 3	Series: Experimental 4	Series: Experimental 5
1	80	5,6894	5,5612	5,2351	5,5320	5,6122
2	90	6,1021	6,0221	6,0212	6,1223	6,0212
3	100	6,8454	6,5212	6,6231	6,5986	6,7545
4	110	7,1221	7,0021	7,1212	6,9851	7,0121
5	120	7,5689	7,4212	7,5655	7,4651	7,5622
Linear Connection Free Member (a)		1,88658	1,80556	0,75242	1,81162	1,70154
Linear connection angle coefficient (b)		0,047790	0,047000	0,057608	0,047290	0,048909
Correlationcoefficient (R)		0,99066	0,99961	0,99314	0,99743	0,99217
Regression equation Y=aX+b		Y=1,88658 X+0,04779	Y=1,80556 X+0,04700	Y=0,75242 X+0,057608	Y=1,81162 X+0,04729	Y=1,70154 X+0,048909

In accordance with the requirements of the 12 edition of GPh, in the joint venture "Remedy group" shell-coated tablets "Sedarem" meet the linearity of the drug in five series, in the field of analysis (from 80 to 120%). The method of analysis for determining the amount of phenolic compounds in shell-coated tablets "Sedarem" meets the requirements of validation on the indicators "Area of analysis" and "Linearity".Validation of analytical methods for determining the amount of " Sedarem" film-coated tablets was carried out. Sedarem shell-coated tablets meet the requirements of the 12 edition of GPh of the indicator "Validation of analytical methods" for quantitative and quantitative methods of valeric acid, phenolic compounds and flavonoids.

Conclusion: Validation analysis of sedatives revealed accuracy, repeatability, linearity, precision, intermediate accuracy, and correlation coefficient.

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