СИНТЕЗ ТА АНАЛІЗ БІОЛОГІЧНО АКТИВНИХ РЕЧОВИН

Рекомендована д.х.н., професором С.І.Мерзлікіним

УДК 615.453.6:615.225.2:54.062:543.42.062

VERIFICATION OF THE QUANTITATIVE DETERMINATION METHOD OF VERAPAMIL HYDROCHLORIDE IN TABLETS

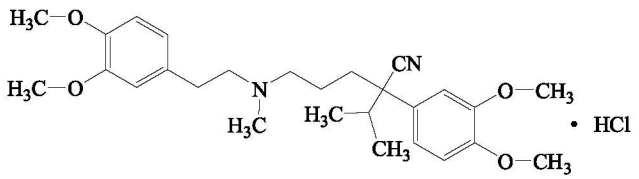
I.L.Komarytsky, N.Yu.Bevz, V.A.Georgiyants

National University of Pharmacy

Verification procedures carried out the quantitative determination of verapamil hydrochloride in tablets by spectrophotometric method. In the process of verification of the verapamil hydrochloride tablet quantative determining were studied some validation characteristics of spectrophotometric quantative determining methods using the standard method: accuracy and convergence. Validational characteristics of the methods do not exceed the critical value of the error (2.4%) and are characterized by qualitative analytical indicators. This method can be correctly reproduced in the laboratory.

Verapamil was synthesized in the late 50's in search of antiadrenergic drugs with the coronary dilator effect. To some extent verapamil has the antiadrenergic action, and it restricts the flow of sympathetic impulses to the blood vessels, heart and other organs. But later (1967) it was shown that the effect of verapamil on the heart (a negative inotropic effect) should be explained by its specific ability to inhibit the passage of calcium ions through the cardiomyocytes. This gave rise to the emergence of a new group of drugs – calcium antagonists.

The chemical structure of verapamil hydrochloride is (2RS)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl)amino]-2-(1-methylethyl)pentannitril hydrochloride (**$C_{27}H_{39}ClN_2O_4$**).



The State Pharmacopoeia of Ukraine and the British Pharmacopoeia recommend to determine verapamil hydrochloride in the substance quantitatively by the alkalimetry method harmonized with the European Pharmacopoeia and direct titration [2, 8, 9].

In medicinal forms the quantitative determination of verapamil hydrochloride is carried out by UV spectrophotometry calculating the content of the active substance by the standard method [8].

Based on this our task was to analyze the metrological characteristics of the quantitative spectrophotometric determination of verapamil hydrochloride tablets and to perform verification of the analytical method by the standard method in the conditions of the control and analytical laboratory with the purpose of further introduction of this method in the second edition of SPhU.

First there was a theoretical calculation of the acceptance criteria analysis methods [1-4]: the maximum total uncertainty method, $\Delta A_s = 2.4\%$, the maximum bias $max\ \delta = 0.77\%$. The placebo contribution to the total value of the background absorption is insignificant and it can be ignored if the ratio is $\delta_{exc} \leq 0.75\%$, the critical $RSD_0\% = 1.27$, the critical value of the correlation index is $R_c = 0.9912$, the critical practical uncertainty free member linear dependence is $a = 3.84$.

Experimental part

When conducting the research the substance of verapamil hydrochloride manufactured by Teva Pharmaceuticals Fine Chemicals S.r.l., batch No. 040 312 (Italy) was used.

The following tablets were analysed: «Verapamil hydrochloride», batch No. 40410, manufacturer – «Borschagovsky Chemical and Pharmaceutical plant» (BChPhP) JSC SPC; «Verapamil-Darnitsa», batch No. BU20611, manufacturer – «Pharmaceutical Company» Darnitsa» JSC; «Lekoptin», batch No. VS7936, manufacturer – Lek Pharmaceutical Company dd, Slovenia, the enterprise of Sandoz company.

The following analytical equipment was used: Evolution 60S spectrophotometer; AXIS ANG200 balance. Reagents, measuring glassware of class A (first class) and excipients meeting the requirements of SPhU were used for the work.

Quantitative determination of verapamil hydrochloride tablets by the standard method was performed by measuring the absorbance of the sample solution (A_x) and the reference solution (A_{st}) with the concentration C_{st}. The concentration C_x of the analyte was calculated by the formula:

$$C_x = \frac{A_i \cdot m_{st} \cdot 5 \cdot 200 \cdot 50 \cdot b}{A_{st} \cdot m_i \cdot 200 \cdot 50 \cdot 5} \cdot \frac{P}{100}.$$

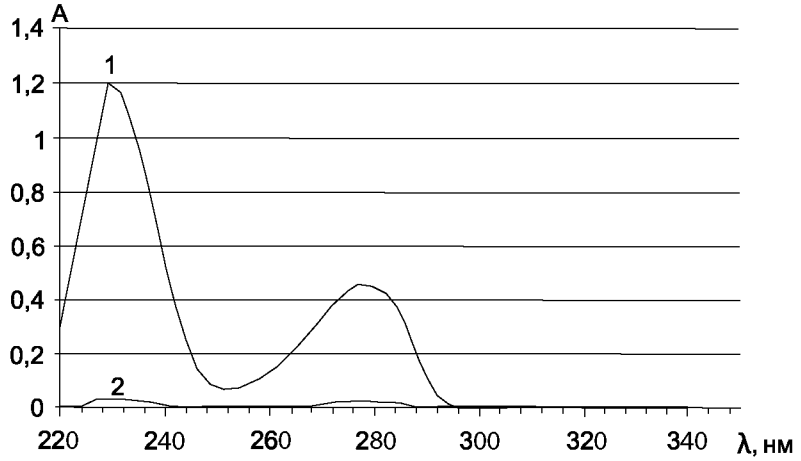


Fig. 1. The ultraviolet absorption spectrum of verapamil PhRS (1) and placebo (2).

The measurements were performed with a 1-cm cell at (20±1)°C under the same conditions with the minimum interval of time.

Results and Discussion

To implement the method we obtained and analyzed the absorption spectrum of the area from 210 to 340 nm, the dependence of the optical density on the concentration of the sample solution in an acceptable range of concentration, and a spectrum of placebo (Fig. 1). The resulting spectrum has the maximum absorption at the wavelength of 278 nm, which also corresponds to the analytical wavelength recommended by the European Pharmacopoeia.

It has been found that the effect of placebo on the total absorption of the drug is

$$\frac{A_{blank}}{A_{st}} \cdot 100 = \frac{0{,}0031}{0{,}465} \cdot 100\% = 0{,}667\% \leq 0{,}75\%.$$

This means that the background absorption is insignificant and is characterized by the appropriate method specificity.

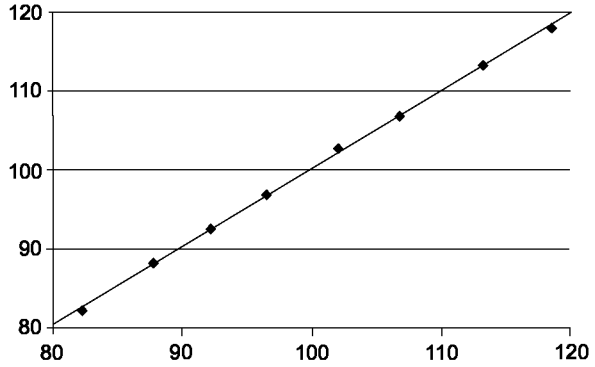
The linearity evaluation was carried out within the whole range of the method application by the standard method. The study of the nature of the optical density on the concentration was performed using 9 model solutions for analysis with accurately weighed concentrations: 80, 85, 90, 95, 100, 105, 110, 115 and 120%.

The results obtained were statistically processed using the least square method according to the requirements of SPhU. The calibration curve was plotted in normalized coordinates (Fig. 2). The mean values of optical density (A_i) were calculated for each of the nine sample solutions. The results were processed by the least square method for the straight line $Y = b \times x + a$. The calculated statistical values b, S_b, a, S_a, S (residual standard deviation) and r (the correlation coefficient) is shown in Fig. 2.

The requirements for the linear dependence in this case are met within the range of the method application (80-120%).

To conduct measurements and calculation of the metrological evaluation of convergence and accuracy of the procedure three absorbance values for the reference solution and 27 optical density values for model solutions were obtained. The actual values (Xi_{act}), the ratios of the average optical density values for each of 27 solutions to the mean absorbance of the reference solution obtaining values $X_i = (C_i/C_{st})\ 100\%$, $Y_i = (A_i/A_{st})\ 100\%$, as well as the value $Z_i = (Y_i/X_i)\ 100\%$, i.e. the concentration found (%) to the concentration introduced, were calculated. The results of the calculations are shown in Table 1.

The application of the standard method makes it possible to offset the errors since the sample preparation



Solutions	1	2	3	4	5
(C_i/C_{st}),%	82.20	87.71	92.26	96.49	102.00
(A_i/A_{st}),%	82.15	87.91	92.51	96.81	102.65
Solutions	6	7	8	9	
(C_i/C_{st}),%	106.76	113.26	118.54	122.55	
(A_i/A_{st}),%	106.81	113.30	118.11	122.64	

$$Y_i = 0.9929 \times X_i - 0.8468$$

The slope ratio of the linear dependence b	0.992943
s_b	0.0069
The constant term of the linear dependence a	0.8468
s_a	0.9516
The residual standard deviation s_r	0.4707
s_r/b	0.47402
The correlation coefficient of the method r	0.9996

Fig. 2. The plot of dependence of the optical density on the concentration of verapamil hydrochloride in normalized coordinates.

Table 1

The analysis of model solutions and their statistical analysis

	Concentration of the components		
	Introduced in % to the concentration of the reference solution $X_i = (C_i/C_{st}) \times 100\%$	Found in % to the concentration of the reference solution $Y_i = (A_i/A_{st}) \times 100\%$	Found in % to the introduced $Z_i = (A_i/A_{st})\ 100\%/(C_i/C_{st})$
Solution 1	82.20	82.15	99.94
Solution 2	87.91	87.91	100.23
Solution 3	92.26	92.51	100.27
Solution 4	96.49	96.81	100.33
Solution 5	102.00	102.65	100.64
Solution 6	106.76	106.81	100.05
Solution 7	113.26	113.30	100.04
Solution 8	118.54	118.11	99.64
Solution 9	122.55	122.64	100.07
Mean, $X\%$			100.134
Relative standard deviation, $s_x\%$			0.316
Relative confidence interval $\Delta\% = t(95\%,8)\ s_x = 1.860 \times s_x$			0.25
Critical value for convergence of the results $\Delta\% \leq$			2.4
Systematic error $\delta = \lvert X - 100 \rvert$			0.134
Criterion of the systematic error insignificance 1) $\delta \leq \Delta/3 = 0.77/3 = 0.257$, if it is not satisfied 1), then $\delta \leq 0.77$			satisfied satisfied
The overall conclusion of the procedure:			correct

Table 2

Stability of the test solution and the reference solution

Solution*	The term of studying stability nt, min					Mean	RSD_t, %	Δ_t, %	max δ, %
	0	15	30	45	60				
Test	0.4653	0.4650	0.4660	0.4657	0.4663	0.46566	0.2362	0.0031	0.77
Reference	0.4650	0.4650	0.4653	0.4657	0.4653	0.46526	0.5712	0.0074	

* The values of the optical density is the average of three measurements of the solution.

and measurement of the optical density of the test solution and the reference solution are carried out under the same conditions in the same cell, which is similarly placed opposite the same compensation cell, on the same spectrophotometer.

Stability of the analytical solution is checked within one hour. The results obtained are shown in Table 2. The statistical evaluation of the effect of time on the test solution meets the acceptance criteria.

Quantitative determination method

Absorption spectrophotometry in the ultraviolet region (2.2.25 standard method).

The test solution. To the accurately weighed tablet powder, which is equivalent to 80 mg of verapamil hyd-

Table 3

The results of the quantitative determination of verapamil hydrochloride tablets

No. of the sample	Manufacturer	A_i	A_{mean}	X_i, mg	X_i, %	Conclusion
1	Darnitsa	0.466 0.465 0.464	0.465	40.14	100.35	conformed
2	BChPhP	0.471 0.468 0.467	0.4687	80.29	100.36	conformed
3	Sandoz	0.472 0.471 0.472	0.4717	40.49	101.23	conformed

rochloride, add 150 ml of 0.01 M solution of hydrochloric acid, shake for 30 minutes, dilute the solution volume with 0.01 M solution of hydrochloric acid to 200.0 ml and filter. Dilute 5 ml of the solution obtained with 0.01 M solution of hydrochloric acid to the volume of 50.0 ml.

Reference solution. Dissolve 80.0 mg of verapamil hydrochloride PhRS in 0.01 M solution of hydrochloric acid and dilute the volume of the solution with the same solvent to 200.0 ml. Dilute 5.0 ml of the solution obtained with 0.01 M solution of hydrochloric acid to the volume of 50.0 ml.

Compensation solution. 0.01 M solution of hydrochloric acid.

The optical density of the test solution and the reference solution is measured at the wavelength of 278 nm with respect to the compensation solution.

Calculate the content of $C_{27}H_{39}ClN_2O_4$ in one tablet, in milligrams, equivalent to the average weight of a tablet based on the declared content of $C_{27}H_{39}ClN_2O_4$ in verapamil hydrochloride PhRS.

The results of the quantitative determination of verapamil hydrochloride are given in Table 3.

CONCLUSIONS

1. Such validation characteristics of the spectrophotometric method for quantitative determination of verapamil hydrochloride tablets as correctness and convergence have been studied using the standard method.

2. Metrological characteristics of the method do not exceed the critical value of the error (2.4%) and are characterized by qualitative analytical indicators. This method can be correctly reproduced in the laboratory.

3. This method is correct regardless of the excipients used in the preparation of the model solution.

LITERATURE

1. *Гризодуб А.И. // Фармаком. – 2006. – №1/2. – С. 34-44.*
2. *Державна фармакопея України. – Х.: РІРЕГ, 2001. – 556 с.*
3. *Державна фармакопея України. – Доп. 1. – Х.: РІРЕГ, 2004. – 520 с.*
4. *Евтифеева О.А., Георгиянц В.А. // Фармаком. – 2007. – №1. – С. 69-81.*
5. *Международная фармакопея / ВОЗ. – 3-е изд. – Женева: ВОЗ, 1995. – Т. 4: Испытания, методы и общие требования. Спецификации для контроля качества фармацевтических препаратов, вспомогательных веществ и дозированных лекарственных форм. – 3-е изд. – 425 с.*
6. *Фармацевтическая химия: Учеб. пособие / Под ред. А.П.Арзамасцева. – М.: ГЭОТАР-МЕД, 2004. – 200 с.*
7. *Borges Ney Carter do C., Mendes Gustavo D., Barrientos-Astigarraga Rafael E. et al. // J. Chromatogr. B. – 2005. – Vol. 827, №2. – P. 165-172.*
8. *British Pharmacopoeia. – London: The Stationary Office, 2001. – Vol. 1-2. – 3199 p.*
9. *European Pharmacopoeia. – 6th ed. – Strasbourg: European Department for the Quality of Medicines, 2008. – 2416 p.*
10. *Ferioli V., Gamberini G. // Chromatographia. – 1997. – Vol. 44, №9/10. – P. 35-37.*

УДК 615.453.6:615.225.2:54.062:543.42.062

ВЕРИФИКАЦИЯ МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ВЕРАПАМИЛА ГИДРОХЛОРИДА В ТАБЛЕТКАХ

И.Л.Комарицкий, Н.Ю.Бевз, В.А.Георгиянц

Проведена верификация методики количественного определения верапамила гидрохлорида в таблетках спектрофотометрическим методом. В процессе верификации методики количественного определения верапамила гидрохлорида в таблетках изучены некоторые валидационные характеристики спектрофотометрической методики количественного определения методом стандарта: правильность и сходимость. Валидационные характеристики методики не превышают критического значения погрешности (2,4%) и характеризуются качественными аналитическими показателями. Данная методика может быть корректно воспроизведена в условиях лабораторий.

УДК 615.453.6:615.225.2:54.062:543.42.062

ВЕРИФІКАЦІЯ МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ВЕРАПАМІЛУ ГІДРОХЛОРИДУ В ТАБЛЕТКАХ

І.Л.Комарицький, Н.Ю.Бевз, В.А.Георгіянц

Проведена верифікація методики кількісного вмісту верапамілу гідрохлориду в таблетках спектрофотометричним методом. У процесі верифікації методики кількісного визначення верапамілу гідрохлориду в таблетках вивчені деякі валідаційні характеристики спектрофотометричної методики кількісного визначення за методом стандарту: правильність та збіжність. Валідаційні характеристики методики не перевищують критичного значення похибки (2,4%) і характеризуються якісними аналітичними показниками. Дана методика може бути коректно відтворена в умовах лабораторій.