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DEVELOPMENT OF THE METHODOLOGY OF THE CHROMATOGRAPHIC DETERMINATION OF VERAPAMILE HYDROCHLORIDE IN MEDICINES

Objective: The objective of this research was to develop more simple, sensitive, accurate and less expensive analytical methods for the determination of verapamil hyrdochloride in medicines by HPLC. Methods: The chromatographic analysis of verapamil hyrdochloride performed on liquid chromatograph Agilent 1290 Infinity II LC System. Results: In developing our technique, column Nucleosil C18 was used, which has several advantages from a number of columns L1 and provides high speed and high efficiency at a lower pressure system. This reduces the number of used mobile phase that according reduces the cost analysis. Selected conditions were isocratic elution with binary mobile phase consisting of methanol, water, acetic acid and triethylamine in the ratio 55 : 44 : 1 : 0.1 for optimum peak symmetry of the active pharmaceutical ingredient. Under this conditions the peak of verapamil elution is about 2 minutes. Besides reducing analysis time achieved by simplifying sample preparation by using methanol as solvent and mobile phase. The proposed method has the advantage over pharmacopoeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time. Conclusion: The results obtained in this research work clearly indicated that the proposed method is rapid, economical, simple, accurate, selective, precise and applicable to the analysis of verapamil hyrdochloride in medicines.

Key words: verapamil hydrochloride; high-Performance liquid chromatography; validation; linearity; accuracy; range of application

INTRODUCTION

Nowadays, the number of medicines in the pharmaceutical industry has greatly increased. Analytical methods of development of active pharmaceutical ingredients and validation plays an important role for pharmaceutical development activities for improving to more accurate, reliable, robust analytical methods and for dicreasing of cost analytical analysis [2]. The object of the study was chosen verapamil. Verapamil hyrdochloride, (2*RS*)-2-(3,4-dimethoxyphenyl)-5-[[2-(3,4-dimethoxyphenyl)ethyl](methyl) amino]-2-(1-methylethyl)pentanenitrile hydrochloride, is a potent dihydropyridine-type calcium channel blocker which is an antihypertensive medicine. Analysis of verapamil hyrdochloride in substance is described in Pharmacopoeia. Chromatographic conditions to determine verapamil hyrdochloride have been shown in Pharmacopoeia, the following chromatographic conditions are column chromatographic categories L1 (with a fixed phase C18) size of 4.6 mm × 125 mm or 150; mobile phase – acetonitrile: 2-aminoheptan: solution A (0.015 M sodium acetate solution containing 33 ml/l acetic acid) (30 : 0.5 : 70); wavelength – 278 nm, flow rate – 0.9 ml/min [7]. Because of creation of the second edition of SPhU and inclusion of articles in the finished products, we have set ourselves the goal to improve to more rapid, simple, selective, more accurate, precise, reliable, less expensive methods of HPLC nalysis of verapamil hyrdochloride in medicines and for analysis of their metabolites in next step of the researches [3].

The aim of work was the development of simple, sensitive, accurate and less expensive analytical methods for the determination of verapamil hyrdochloride in medicines by HPLC.

MATERIALS AND METHODS

The objects of the study were tablets "Verapamil Darnitsa" (Ukraine). The chromatographic analysis of verapamil hyrdochloride performed on liquid chromatograph Agilent 1290 Infinity II LC System.

Chromatography is performed on liquid chromatograph with spectrophotometric detector under the following conditions:

- column Nucleosil C18 4.6 × 150 mm with a particle size of 5 microns;
- mobile phase: *methanol R – water R – acetic acid R – triethylamine R (55 : 44 : 1 : 0.1)*;
- the rate of mobile phase: 0.8 ml/min;
- column temperature: 250°;
- detection wavelength: 280 nm.

Preparation of Test Solution

Test solution. To 62.5 mg powder pounded tablets, add 10 ml of *methanol R*, shake in ultrasonic bath for 10 minutes and add *methanol R* to the volume of 20.0 ml. Filter through a membrane filter with a pore size of 0.45 microns, discarding the first 5 ml of filtrate. 5.0 ml of the resulting filtrate adjusted to 25.0 ml of solvent.

Preparation of SS Solution

20.0 mg of verapamil hyrdochloride SPhU dissolve in *methanol R* and dilute with the same solvent to 20.0 ml volume. 5.0 ml of the resulting solution adjusted to 25.0 ml of solvent.

Validation of the method was carried out in accordance with the requirements of the SPhU [5, 6].

RESULTS AND DISCUSSION

For elaboration of the method the chromatograms of the standard solution of verapamil hyrdochloride (Fig. 1) and the test solution of verapamil hyrdochloride (Fig. 2), as well as the dependence of the intensity peaks on the retention time were obtained and analysed.

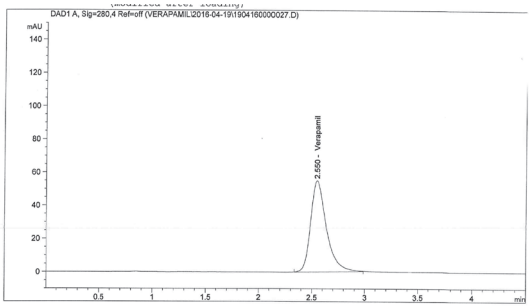
The results of the analysis are considered reliable if the requirement of the System Suitability Test are performed. The chromatographic system is considered suitable if the following conditions are performed:

- Relative standard deviation calculated peak area for verapamil hyrdochloride should be not more than 1.0 %;
- Symmetry factor calculated on the peak of verapamil hyrdochloride should be less than 2.

The content of verapamil hyrdochloride (X) in one tablet, in milligrams, calculated by the formula:

$$X = \frac{S_i \cdot m_o \cdot b \cdot P}{S_o \cdot m_i \cdot 100},$$

where: S_i – average of the peak areas of verapamil hyrdochloride, calculated from the chromatogram of the test solution; S_0 – average of the peak areas of verapamil hyrdo-



External Standard Report

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Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

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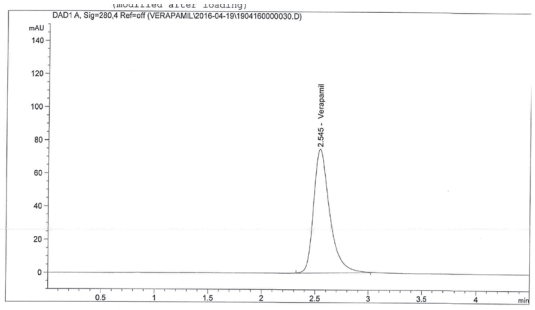
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
2.550	BB	567.66144	2.54508e-4	1.44474e-1		Verapamil

Totals : 1.44474e-1



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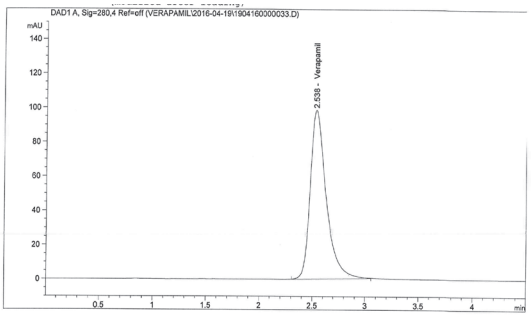
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
2.545	BB	781.84680	2.54536e-4	1.99008e-1		Verapamil

Totals : 1.99008e-1



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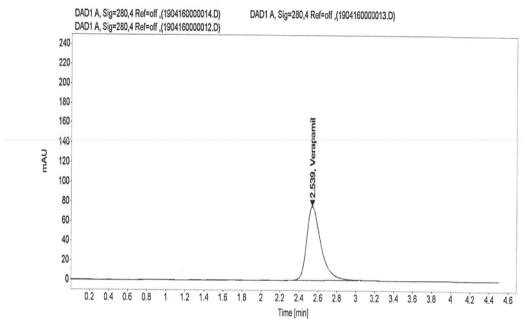
Dilution : 1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: DAD1 A, Sig=280,4 Ref=off

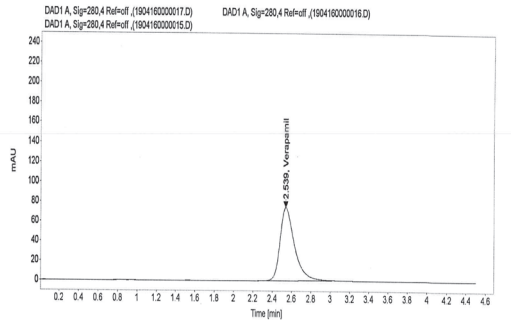
RetTime [min]	Type	Area [mAU*s]	Amt/Area	Amount [ng/ul]	Grp	Name
2.538	BB	1038.05457	2.54555e-4	2.64242e-1		Verapamil

Totals : 2.64242e-1

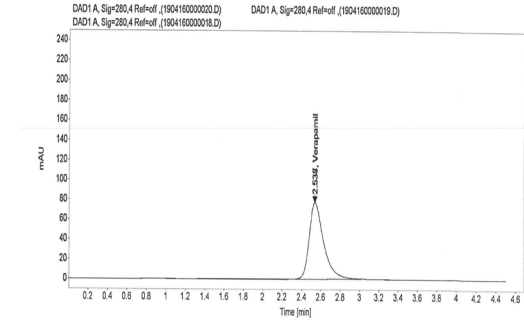
Fig. 1. *HPLC chromatograms of the standard solution of verapamil hyrdochloride in the terms of the quantification of verapamil hyrdochloride in tablets*



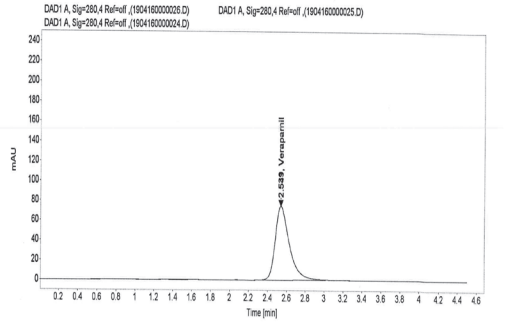
Name	RT [min]	Area	TailFactor	Peak_Resolution_EP
Verapamil	2.539	790.145	1.49367	
	2.539	790.863	1.53247	
	2.539	792.124	1.53896	
	Sum	2373.132		
	Average	791.044		
	RSD	0.127		



Name	RT [min]	Area	TailFactor	Peak_Resolution_EP
Verapamil	2.539	777.155	1.51282	
	2.539	776.881	1.49367	
	2.539	776.192	1.51282	
	Sum	2330.228		
	Average	776.743		
	RSD	0.064		



Name	RT [min]	Area	TailFactor	Peak_Resolution_EP
Verapamil	2.537	807.351	1.47500	
	2.538	807.796	1.46296	
	2.538	804.743	1.51923	
	Sum	2419.890		
	Average	806.630		
	RSD	0.204		



Name	RT [min]	Area	TailFactor	Peak_Resolution_EP
Verapamil	2.539	773.703	1.50641	
	2.539	774.159	1.50000	
	2.541	774.644	1.51282	
	Sum	2322.507		
	Average	774.169		
	RSD	0.061		

Fig. 2. *HPLC chromatograms of the test solution of verapamil hyrdochloride in the terms of the quantification of verapamil hyrdochloride in tablets*

chloride, calculated with the standard solution chromatogram; m_0 – mass of the sample SPhU verapamil hyrdochloride, in milligrams; m_i – mass of the powder pounded tablets, in milligrams; P – content of the main substance in SPhU verapamil hyrdochloride as a percentage; b – average of weight tablets in milligrams.

In developing this technique, column Nucleosil C18 was used, which has several advantages from a number of columns L1 and provides high speed and high efficiency at a lower pressure system. This reduces the number of used mobile phase that according reduces the cost analysis.

Selected conditions were isocratic elution with binary mobile phase consisting of methanol, water and acetic acid and triethylamine in the ratio 55 : 44 : 1 : 0.1 for optimum peak symmetry of the active pharmaceutical ingredient. Detection was performed at 280 nm. Under this conditions the peak of verapamil elution is about 2 minutes.

The method has the advantage over pharmacopoeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time.

According to the requirements of the SPhU, methods of quantitative determination of medicines must be vali-

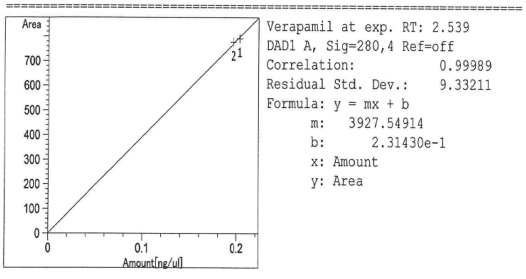


Fig. 3. *Calibration curve for HPLC chromatographic determination of verapamil hyrdochloride in tablets and metrological characteristics of linearity*

Table

THE RESULTS OF TESTING THE QUANTITATIVE DETERMINATION OF VERAPAMIL HYRDOCHLORIDE ON THE ACCURACY BY METHOD HPLC

Solution	Put (solution concentration), mg/ml	Found (concentration solution), mg/ml	The relation found to input, Z, %
RS1	0.203	–	–
RS2	0.196	–	–
MS 70 %	0.146	0.144	98.6
MS 100 %	0.196	0.199	101.5
MS 130 %	0.259	0.264	101.9
The average			100.7
The relative standard deviation, Sz %			0.032
Relative confidence interval, Δz %			0.06
The critical value for convergence results Δz % ≤ 3.2			Correct
The criterion of statistical insignificance systematic error $\delta\% = \lvert \bar{Z}\text{-}100 \rvert \le \Delta_z / \sqrt{n}$	≤ 0,20	Correct	
The overall conclusion of the procedure		Correct	

dated. We have studied the following validation characteristics: linearity, accuracy and range of application [1, 4].

Evaluation of linearity was performed on the entire range of application of the method using standard method. The study of dependence of absorbance on the concentration was conducted using model solutions of the samples. The results obtained were statistically processed by the least squares method according to the requirements of the SPhU. For each of test solutions the average value of the peak area were calculated. The results obtained were processed by the least squares method for line $y = m \times x + b$ and metrological characterictsare are shown in Fig. 3.

Requirements for the parameters of the linear dependence in this case are carried out within the whole range of the method application (70-130 %). Accuracy and convergence were studied by "put-found" on standard solutions of verapamil hyrdochloride.

Prepared 3 models of verapamil hydrochloride solutions API:

Name of solution	Sample, mg
MS 70 %	14.6
MS 100 %	19.6
MS 130 %	25.9

Prepared 2 reference solution:

Name of solution	Sample, mg
RS 1	20.3
RS 2	19.6

Model solutions were prepared according to the procedure completely repeating the procedure for preparing the test solution. By comparing the two solutions for each analyte built calibration graph (level 1-2, including all parallel injection and specifying the appropriate concentration reference solution), passing through zero. For calibration schedule, each analyte concentration was calculated corresponding model solution (Table).

To measure and calculate the metrological evaluation of convergence and accuracy of the method were obtained. The actual values the ratio of the average values of peak for each solutions were calculated to the mean peak area of the reference solution, the values *Xi = (Ci/Cst) × 100 %, Yi = (Yi/Yst) × 100 %*, as well as the value *Zi = (Yi/Xi) × 100 %*, the concentration found in % to the concentration introduced were determined. The calculation results are shown in Table. To assess the intermediate precision the relative confidence interval for 5 parallel measurements of the quantitative content of substances, which should be less than the maximum permissible uncertainly analysis results (Dz % £ 3.2 %), was used. Tests were carried out using one batch of the drug by different drug analysts on the same chromatograph in different days using different measuring vessels.

CONCLUSIONS

In conclusion, the developed method has the advantage over pharmacopoeial method due to speed and ease of preparation of the mobile phase and reduced chromatography time and cost of analysis. The results obtained in this research work clearly indicated that the proposed HPLC method is rapid, economical, simple, accurate, selective, precise and applicable to the analysis of verapamil hyrdochloride in medicines.

REFERENCES

1. ICH Topic Q2 (R1) Validation of Analytical Procedures: Text and methodology.
2. Logoyda L. Development and validation of new methods of analysis for the determination of different natural and synthetic original active pharmaceutical ingredients in medicines. Duphat 2015; 48.
3. Logoyda L. Development of methods for identification of calcium channel blockers in medicines / L. Lo-

goyda, D. Korobko, S. Saprun // Int. J. Res. Ayurveda Pharm. – Mar.-Apr. – 2016. – Vol. 7 (2). – P. 88-91.
4. Logoyda L. Validation of chromatographic methods of analysis for the determination of active pharmaceutical ingredients in different medicines. PharmaSchool association for pharmaceutical development and scientific research, 2016. – 34 p.
5. State Pharmacopoeia of Ukraine: in 3 Vol. / State Enterprise "Ukrainian Scientific Center pharmacopoeia quality medicines". – 2-nd ed. – Kharkiv: State Enterprise "Ukrainian Scientific Center of Quality pharmacopoeia of medicines", 2014. – Vol. 2. – 724 p.
6. State Pharmacopoeia of Ukraine / State Enterprise "Scientific and Expert Centre pharmacopoeia". – 1-st ed. – Appendix 2. – Kharkiv: State Enterprise "Ukrainian Scientific Center of Quality pharmacopoeia of medicines", 2008. – 620 p.
7. "United States Pharmacopoeia (USP). Medicare Prescription Drug Benefit Model Guidelines Source Information". www.nlm.nih.gov

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РОЗРОБКА МЕТОДОЛОГІЇ ХРОМАТОГРАФІЧНОГО ВИЗНАЧЕННЯ ВЕРАПАМІЛУ ГІДРОХЛОРИДУ В ЛІКАРСЬКИХ ЗАСОБАХ

Метою даного дослідження була розрока більш простих, чутливих, точних і менш дорогих методів визначення верапамілу гідрохлориду в лікарських засобах за допомогою високоефективної рідинної хроматографії. Хроматографічний аналіз верапамілу гідрохлориду проводили на рідинному хроматографі Agilent 1290 Infinity II LC System. При розробці нашої методики використовувалася колонка Nucleosil C18, яка має ряд переваг серед колонок L1 і забезпечує високу швидкість і високу ефективність при більш низькому тиску системи. Це зменшує кількість використовуваної рухомої фази, що відповідно знижує аналіз витрат. Нами були підібрані умови ізократичного елюювання з рухомою фазою, що складалася з метанолу, води, оцтової кислоти та триетиламіну у співвідношенні 55 : 44 : 1 : 0.1 для досягнення оптимальної симетрії піку активного фармацевтичного інгредієнта. За даних умов пік верапамілу елююється близько 2 хвилин. Крім цього, скорочення часу аналізу досягалося за рахунок спрощення умов пробопідготовки завдяки використанню в якості розчинника метанолу та рухомої фази. Запропонований метод аналізу має перевагу в порівнянні з фармакопейним методом завдяки швидкості і легкості приготування рухомої фази і скорочення часу хроматографування. Результати, отримані в роботі, чітко свідчать, що запропонований метод є швидким, економічним, простим, точним і підходить для визначення верапамілу гідрохлориду в лікарських засобах.

Ключові слова: верапамілу гідрохлорид; високоефективна рідинна хроматографія; валідація; лінійність; точність та прецизійність, діапазон застосування

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РАЗРАБОТКА МЕТОДОЛОГИИ ХРОМАТОГРАФИЧЕСКОГО ОПРЕДЕЛЕНИЯ ВЕРАПАМИЛА ГИДРОХЛОРИДА В ЛЕКАРСТВЕННЫХ СРЕДСТВАХ

Целью данного исследования была разработка более простых, чувствительных, точных и менее дорогих методов определения верапамила гидрохлорида в лекарственных средств с помощью высокоэффективной жидкостной хроматографии (ВЭЖХ). Хроматографический анализ верапамила гидрохлорида проводили на жидкостном хроматографе Agilent 1290 Infinity II LC System. При разработке нашей методики использовалась колонка Nucleosil C18, которая имеет ряд преимуществ среди колонок L1 и обеспечивает высокую скорость и высокую эффективность при более низком давлении системы. Это уменьшает количество используемой подвижной фазы, что соответственно снижает анализ затрат. Нами были подобраны условия изократического элюирования с подвижной фазой, состоящей из метанола, воды, уксусной кислоты и триэтиламина в соотношении 55 : 44 : 1 : 0.1 для достижения оптимальной симметрии пика активного фармацевтического ингредиента. При данных условиях пик верапамила элюируется около 2 минут. Кроме этого, сокращение времени анализа достигалось за счет упрощения условий пробоподготовки благодаря использованию в качестве растворителя метанола и подвижной фазы. Предложенный метод анализа имеет преимущество по сравнению с фармакопейным методом благодаря скорости и легкости приготовления подвижной фазы и сокращения времени хроматографирования. Результаты, полученные в работе, четко свидетельствуют, что предложенный метод является быстрым, экономичным, простым, точным и подходит для определения верапамила гидрохлорида в лекарственных средствах.

Ключевые слова: верапамила гидрохлорид; высокоэффективная жидкостная хроматография; валидация; линейность; точность и прецизионность; диапазон применения

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