UDC 542:54.084

APPLICATION OF LIQUID CHROMATOGRAPHY FOR "LOSARTAN POTASSIUM" HYPOTENSITIVE DRUG QUALITY CONTROL

L.A. Onuchak, M.V. Vasilyeva
Samara National Research University named after Academician S.P. Korolyov, Russia

---

The authors of this paper have developed a method for determining the hypotensive pharmaceutical substance of losartan potassium and its normalized impurity in a substance and in a tablet form using reversed-phase high-performance liquid chromatography (RP HPLC) has been developed. The quantitative determination of losartan potassium and its impurity the external standard method was applied. The experimental data obtained with validation have confirmed the selectivity, precision, correctness and sensitivity of the proposed method. The presence of losartan potassium in medicinal agents can also be confirmed by reversed-phase thin-layer chromatography (TLC) using a mixture of phosphate buffer (pH = 3.6) eluent – acetonitrile of 60: 40% composition.

Key words: *receptor, hypotensive drug, metabolite, chromatographic experiment.*

Introduction. Currently, disease of the cardiovascular system is the most widespread and serious one in the world, moreover it is the leading cause of death worldwide. In connection with this, cardiovascular drugs (LS) of different classes presently are widely used. A special place among them is occupied by antihypertensive drugs. Angiotensin II receptor antagonists are one of the new most dynamically developing classes of antihypertensive drugs.

Losartan (Fig. 1) is the most known non-peptidic blocker of angiotensin II receptors.

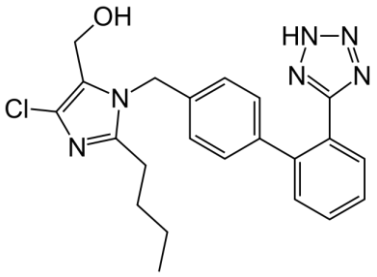


Figure 1. The structural formula of losartan

Blocking of the aforesaid receptors, losartan prevents and eliminates the vasoconstrictive effect of angiotensin II. This drug is characterized by a prolonged action (≥24 h), which is due to the formation of its active metabolite. Losartan is prescribed for patients with arterial hypertension, cardiac insufficiency, atrial fibrillation, patients with kidney pathology and who underwent myocardial infarction.

It is important to note that losartan prevents the development of myocardial hypertrophy, lowers the pressure in the pulmonary vessels, increases exercise tolerance in patients with heart failure. In order to achieve better solubility in the bio-environments and bioavailability, losartan is released in the form of potassium salt - "losartan potassium".

Production of losartan potassium in the Russian Federation is based on the use of foreign substances. Analytic control of substances and products made from them includes qualitative and quantitative analysis of both the main active substance and impurities. Despite the widespread use of losartan potassium in medical practice, in the Pharmacopeia of the Russian Federation [1] there are no regulatory methods for the analysis of this drug. In the American (USP) [2] and European (EP) [3]

pharmacopoeia, various methods (IR and UV spectrometry, potentiometric titration, high-performance liquid chromatography) are proposed to control the main active substance and impurities in the substance, which significantly increases the duration of analysis.

The aim of the work is the development of methods for determining the authenticity and quality of the drug "losartan potassium" using columnar (HPLC) and thin-layer (TLC) reversed-phase liquid chromatography.

Experiment. Objects of analysis, standard samples and reagents. The following have been used as objects of analysis: losartan tableted preparation (25 mg, Pranapharm Ltd., Russia), losartan potassium substance (San Pharmaceutical Industries LTD, India). Standard samples of potassium losartan and losartan impurities C were used to prepare standard and quantitative solutions in accordance with the US Pharmacopoeia (USP RS) catalog.

The following reagents have been used in this work: potassium phosphate monosubstituted (reagent-grade, produced by "Reactiv"), orthophosphoric acid (reagent-grade, production of "Reactiv"), acetonitrile (HPLC-grade, production of "Reactiv"), distilled water.
A standard solution was used to determine the quantitative content of the main component (potassium losartan of 0.4 mg/ml concentration).

To determine the impurity content, a standard solution of impurity C (impurity concentration C = 0.003 mg/ml) was used. To select the optimal conditions for chromatographic separation of losartan and its impurities, a standard solution of a two-component model mixture was used (concentration of losartan potassium - 1 mg/ml, concentration of losartan impurity - 0.003 mg/ml).

Sample preparation. To determine the quantitative content of the main component, the exact batch of powder of crumbled tablets, or the exact batch of the substance equivalent to 10 mg of potassium losartan, was placed in a 25 ml volumetric flask, 15 ml of distilled water was added, processed in an ultrasonic bath for 15 minutes, cooled to room temperature . The volume of the solution was adjusted to the mark with the same solvent, mixed, filtered through a membrane filter of 0.45 μm mesh, discarding the first portions of the filtrate (concentration of potassium losartan was 0.4 mg/ml).

In order to determine the quantitative impurity content, an accurate sample of the powder of crushed tablets or a substance equivalent to 25 mg of potassium losartan was placed in a 25 ml volumetric flask, then 15 ml of distilled water was added, mixed in an ultrasonic bath for 15 minutes, cooled, the volume of the solution was adjusted with the same solvent up to the mark, mixed and filtered through a membrane filter with 0.45 μm pore diameter, discarding the first portions of the filtrate (concentration of potassium losartan is about 1 mg/ml).

Chromatographic experiment. A liquid chromatograph "Knauer" was used with a UV detector, a thermostat of "Jet Stream II Plus" columns enabling elution in the temperature range 4 - 90^0C. A steel chromatographic column (250x4.6mm) filled with "Diaspher C18" sorbent of 5μm granulation ("BioChimMac ST", Russia) was used. The temperature of the column was 30^0C. Prior to each analysis, the column was washed with eluent for 15 minutes. Chromatography was carried out both in the isocratic and in the gradient elution modes. As eluents, mixtures of phosphate buffer pH 2.3 and acetonitrile with an acetonitrile concentration of 30-76% were used.

A chromatographic experiment in thin layer chromatography was carried out using reversed phase plates of Merk TLS aluminium sheets RP-18 F_{254} with an eluent of "phosphate buffer-acetonitrile" of **60:40%** v/v and pH of the original buffer was 3.6. Registration of the chromatogram on plates was carried out using the program "Sorbfil Videodensitometr TLC Quantitative Evaluation" (V. 1.7.0.216) ("Sopolymer", Russia).

The discussion of the results. Losartan or (2-butyl-4-chlorine-1 - {[2 - (1H-tetrazolium-5-yl) biphenyl-4-yl] methyl} -1H-imidazole-5-carbaldehyde ($C_{22}H_{21}ClN_6O$, M_r = 420,89) is a sufficiently hydrophobic compound and poorly soluble in aquatic environment. Therefore, both in substances and in finished drugs, losartan is in the form of potassium losartan. The structural formula of potassium losartan is potassium salt of 2-butyl-4-chlorine- 1 [p- (O-1H-tetrazol-5-yl) phenyl) benzyl] imidazole-5-methanol ($C_{22}H_{22}ClKN_6O$, M_r = 461.0) is shown in Fig. 2

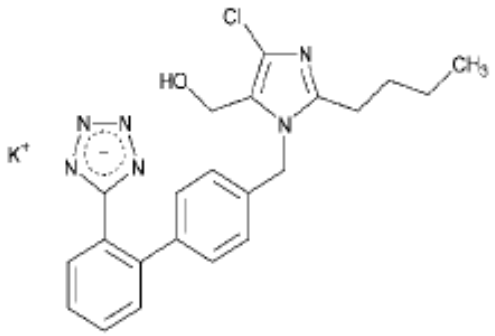


Figure 2. The structural formula of potassium losartan

Lozartan acts as an acid in the formation of salt with potassium. Since the chromatographic analysis was carried out in an acid medium, then dissociation of the salt in a water-acetonitrile medium leads to the formation of a predominantly molecular form of losartan. In this case, the potassium ions on the chromatogram are not recorded, since they do not absorb in the UV region of the spectrum.

In the isocratic mode of elution (30^0C), the effect of the composition of the mobile phase "phosphate buffer (pH = 2.50) - acetonitrile" on the retention of losartan on the nonpolar sorbent octadecylsilicagel C_{18} has been studied. The concentration of acetonitrile was varied from 40 to 76% v/v, which corresponds to a change in the mole fraction of this organic modifier in the mobile phase from X_M = 0.19 to X_M = 0.52.

Figure 3 shows the dependence of the logarithm of the retention factor (*lgk*) of losartan from the logarithm of the mole fraction of acetonitrile (lgX_M) in the eluent.

The linear dependence of *lgk* of lgX_M (R^2 = 0.974) indicates that the sorption of losartan on octadecylsilicagel under conditions of RP HPLC is described by the Snyder-Sochevinsky displacement model [4]:

$$lgk = a\text{-}n'X_M \quad (1)$$

where *n'* is the ratio of the areas of the sorbate molecule (losartan) and the most highly sorptive component of the mobile phase (acetonitrile) in the adsorption layer, and ***a*** is an empiric coefficient (-1.22).

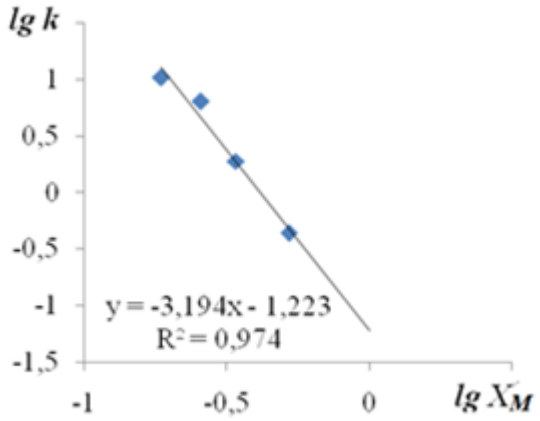


Figure 3. Dependence of the logarithm of the retention factor on the logarithm of the mole fraction of acetonitrile in the mobile phase "phosphate buffer (pH = 2.50) - acetonitrile": the column "Diaspher C18" (250x4.6 mm), 30^{0}C

For losartan, n' = 3.19, therefore, according to experimental data its molecule under competitive adsorption is capable to displace three molecules of an organic modifier from the surface of a nonpolar sorbent.

The Scott-Kuchera sorption model [4] is characterized by a linear dependence

$$1/k = A + BX_M \qquad (2)$$

where A and B are empiric coefficients.

Both models of displacing (competitive) sorption do not take into account associative and solvation effects in the polar mobile phase.

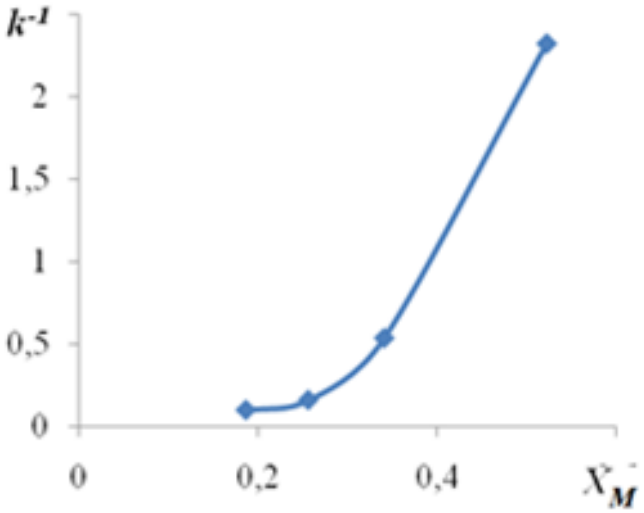


Figure 4. Dependence of the inverse retention factor on the mole fraction of acetonitrile in the mobile phase "phosphate buffer (pH = 2.50) - acetonitrile": the column " Diaspher C18" (250x4.6 mm), 30^{0}C

Figure 4 shows the *1/k* dependence of X_M for potassium losartan, from which it follows that with an increase in the acetonitrile content ($X_M > 0.3$), a rather sharp increase of the slope angle occurs which indicates the association of losartan molecules with acetonitrile molecules in mobile phase [4].

When developing conditions for the qualitative and quantitative determination of potassium losartan and its impurities in substances and tableted dosage forms, it is necessary to meet the requirements designed for carrying out examination of drugs [5]: the methodology should provide indicators of the efficiency of the chromatographic column; the asymmetry factor of the peaks of the main components and impurities should be minimized; a complete resolution of the peaks of the main components and impurities should be ensured; the relative deviation of peak areas should not exceed 5.0%; the limit of quantitative determination of impurities should not exceed the maximum admissible content of impurities in the corresponding test solution.

When conducting a chromatographic experiment in the isocratic mode, using the mobile phase "phosphate buffer (pH = 2.50) - acetonitrile" of various compositions, the separation of the peak of losartan from the peak of its normalized impurity C-2-butyl-5-chlorine-1- { [2 - (1H-tetrazol-5-yl) [1,1 (bifinyl) - 4-yl] methyl}-1-H-imidazole-4-methanol ($C_{22}H_{23}ClN_6O$).

In this connection, various regimes of the gradient supply of the eluent to the chromatographic column have been studied. Gradient elution was carried out using the eluents "phosphate buffer-acetonitrile, 30% v/v" (A) and "acetonitrile" (B). The gradient of the mobile phase composition is shown in Table 1. The flow rate of the mobile phase was 1.0ml/min. The volume of the introduced sample was 20 μm. Detection was carried out at λ = 230nm.

Table 1.

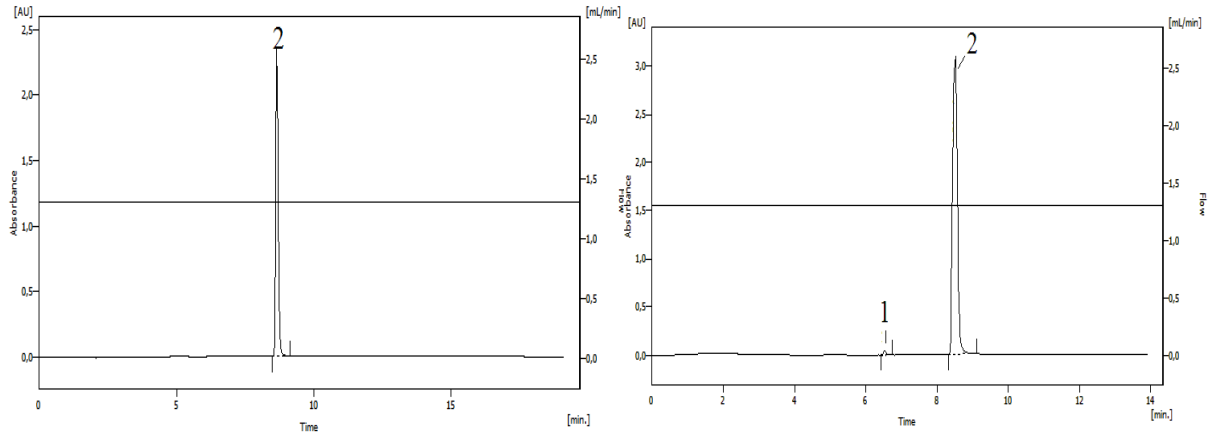
Gradient of the mobile phase composition (30^0C)

Eluent A (phosphate buffer-acetonitrile 70: 30% v/v)	Eluent B (acetonitrile), 100%	Time, min
90	10	0
10	90	15
90	10	30

The elution mode presented in Table 1 allows not only to separate losartan and impurity C, but also to return the eluent composition to the initial one for the purpose of reanalysis. Figure 5 shows chromatograms of the losartan substance (a), as well as a model mixture of the losartan standards and losartan impurities C (b) impurities, obtained under the selected conditions.

In spite of the fact that losartan (*logP* = 5.08) and losartan impurity C (*logP* = 4.90) have close values of lipophilicity and a chemically similar structure of molecules, it was possible to completely separate the peak of losartan potassium from the peak of impurity C, and also to achieve sufficiently good peak asymmetry. The order of the peaks is consistent with the increase in the lipophilicity of the molecules of these analytes.

It can be seen from Fig. 5 that the peaks corresponding to losartan and impurity C are well separated, have small asymmetry and slight erosion, which enables carrying out rapid analysis (less than 10 min) of the drug "losartan potassium". Chromatographic characteristics obtained using the developed conditions are presented in Table 2.



a) b)

Figure 5. Chromatograms of *losartan* potassium (substance) (a) and mixture of potassium losartan standards and impurity C (b): " Diaspher C18", 250x4.6mm, V_{probe} = 10 µl, λ = 230 nm, 30^{0}C; eluent "phosphate buffer (pH = 2.50) - acetonitrile", gradient supply of eluent, 1.5 ml/min; 1 - admixture C; 2 - losartan potassium

Table 2.

Chromatographic characteristics of the components of the model mixture containing potassium losartan and impurity C

Substance	Concentration, mg/ml	t_R, minute	Efficiency of the column T.T.(N)	Asymmetry of the peak	Signal/noise ratio
Losartan (peak 2)	1	8,5	23000	1,50	-
Losartan impurity C (peak 1)	0,003	6,5	15000	1,00	90

It can be seen from the above presented data that the chromatographic peaks have low asymmetry parameters (A≤1.5), and the sensitivity of the method is sufficient for the quantitative determination of the impurity in the tablet composition (signal to noise ratio for the impurity peak is > 20). The number of theoretical plates for losartan is N = 23000 and N = 15000 for impurity C, which indicates the high efficiency of the column.

The validation of the methods for the qualitative and quantitative determination of potassium losartan and its impurity C in tablets using the HPLC method was performed by the following characteristics: selectivity, linearity, accuracy, precision, detection limit, limit of quantitative detection, sample transfer [6-9]. Data obtained during validation, satisfy these requirements, confirm the selectivity, precision, correctness and sensitivity of the proposed methods (Table 3).

Table 3.

Results of validation of methods for the analysis of potassium losartan (RP HPLC)

Parameter of validation	Criteria of acceptability	Obtained values
Specificity	Losartan and impurity peaks should not overlap. Resolution between peaks of losartan and losartan impurity C should be not less than 2,0.	9,00

Linearity	Coefficient of correlation ≥ 0,980	0,996 (ident.) 0,999 (not ident.)
Convergence	Run 1: Coefficient of variation ≤ 5,0% (n≥6); Standard deviation for ident. impurity ≤ 0,02 %	 3,38% 0,00506%
Intermediate precision	Run 2 (obtained by another researcher in another workday): Coefficient of variation ≤ 5,0%; Standard deviation for ident. impurity ≤ 0,02 %; Maximum deviation of the average value ≤ 10,0% For both runs (referred to the maximum value); F-criterion ≤ 5,05; t- criterion < 2,23	 4,30% 0,00694 % 4,52%(1 run) 6,17% (2 run) 1,89 0,60
Accuracy	Response factor: The average value 95,0 – 105,0%; Coefficient of variation ≤ 5,0%; Confidence interval should include 100% of the value	 101,53% (ident.) 100,32% (not ident.) 2,03% (ident.) 1,82% (not ident.) ±4,89 (ident.) ±4,32 (not ident.)
Detection limit	≤ 0,3 % (ident.) ≤ 0,3 % (not ident.)	0,04% 0,06%
Quantification limit	≤ 0,3 % (ident.) ≤ 0,3 % (not ident.)	0,12% 0,19%

The results of quantitative determination of potassium losartan and its admixture in tableted drug form ("LOZARTAN", 25 mg, "PRANAPHARM", Russia, Samara) are presented in Table 4.

Table 4.

Results of quantitative determination of potassium losartan and admixture

Tablets «LOSARTAN», 25 mg («PRANAPHARM), lot # 11114	Losartan	Losartam potassium	23,10 – 26,90 mg	25,44±0,23 mg
		Losartan admixture C	Not more 0,30%	0,19%±0,02%

Table 4 shows that the results obtained fit well into the norms presented in the quality certificates of this drug.

At present, thin-layer chromatography with densitometric detection of zones and computer data processing has become quite accurate, highly sensitive and does not require the use of sophisticated analytical equipment. Although the HPLC method is mainly used to determine the main component and impurities in drugs, the economical TLC method is often used to establish the presence of a major component of drugs in substances and dosage forms.

For the first time we have proposed to apply the reversed-phase TLC method to determine the potassium losartan in the drug. For this purpose, the eluent compositions were used, which were used under RP HPLC conditions. By varying the composition of the "phosphate buffer-acetonitrile" eluent and the pH of the initial buffer, it was found that the eluent "phosphate buffer-acetonitrile" of 60:40% v/v is most suitable for determining potassium losartan. with the pH of the original buffer 3.60. The chromatogram of potassium losartan, obtained by reversed-phase TLC, is shown in Fig. 6.

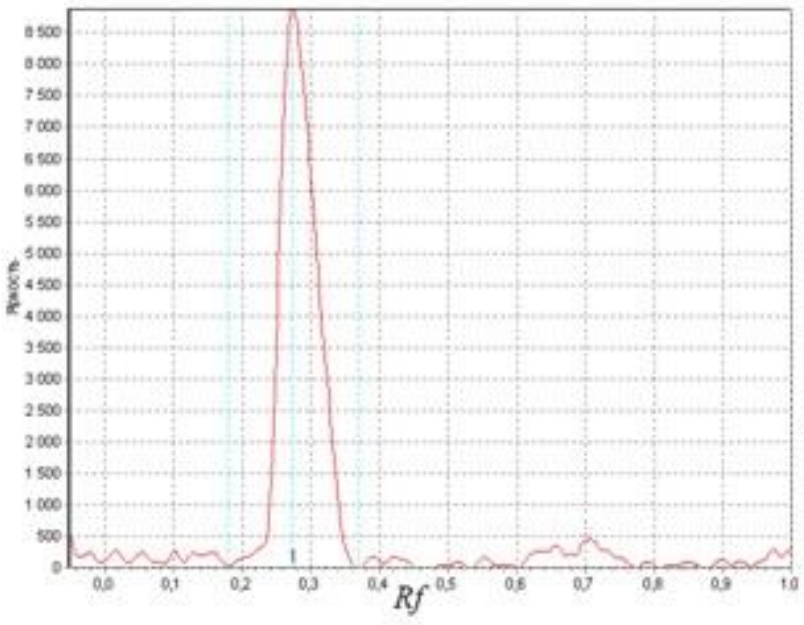


Figure 6. Chromatogram of the potassium losartan substance: Merk TLS aluminum sheets RP-18 F_{254}; mobile phase "phosphate buffer (pH = 3.60) - acetonitrile" of composition 60: 40% v/v. Under the proposed analysis conditions, the value of the delay factor R_f = 0.28 of potassium losartan is a qualitative characteristic of this drug compound. The chromatographic peak has a low asymmetry index (A = 1.95) and small erosion

Conclusion

The procedure for determining potassium losartan and its normalized impurity in a substance and in tableted form by RP HPLC method has been developed and validated. The conditions of analysis are optimized in such a way that in a single cycle of analysis, a qualitative and quantitative analysis of both the main component and the impurity is carried out. For a quick assessment of the presence of potassium losartan in drugs, the TLC method has been proposed.

Acknowledgements

Financial support from the Ministry of Education and Science of the Russian Federation (grant 4.5883. 2017/8.9) is gratefully acknowledged.

References

1. Государственная фармакопея Российской Федерации. Москва, Научный центр экспертизы средств медицинского применения, 2008. 704 с.
2. Фармакопея США. Национальный формуляр. 2 тома. М.: ГЕОТАР-Медиа, 2009. 1720 с.
3. Европейскаяфармакопея, 6 изд. European Directorate for the Quality of Medicines & HealthCare, 2010, 4546 с.
4. Шатц В.Д., Сахартова О.В. Высокоэффективная жидкостная хроматография. Рига: ЗИНАТНЕ. 1988. 390 с.
5. Руководство по экспертизе лекарственных средств. т. 2. / Под ред. Миронова А.Н. и кол. Москва, ГрифиК, 2013, 280 с.
6. Багирова В.Л., Краснюк И.И., Сбоев Г.А. Дженерики: требования, регистрация, использование // Ремедиум. 2004. №3. С. 34-41.

7. Валидация аналитических методик для производителей лекарственных средств: типовое руководство предприятия по производству лекарственных средств. / под ред. Береговых В.В. М.: «Литерра», 2008. 132 с.
8. Об обращении лекарственных средств: Федеральный закон Российской Федерации от 12 апреля 2010 г. N 61-ФЗ.
9. Руководство по валидации методик анализа лекарственных средств (методические рекомендации) / Под ред. Н.В. Юргеля и др. М.:«Спорт и Культура - 2000» 2007. 192 с.

References

1. State Pharmacopeia of the Russian Federation. Moscow, Scientific Center for Expertise of Medical Applications, 2008. 704 p.
2. USA Pharmacopeia. National formulary. 2 volumes. M.: GEOTAR-Media, 2009. 1720 p.
3. European Pharmacopoeia, 6th Edition, European Directorate for the Quality of Medicines & HealthCare, 2010, 4546 p.
4. Shatz V.D, Sakhartova O.V. High performance liquid chromatography. Riga: ZINATNE. 1988. 390 p.
5. Guidelines for the examination of medicines. 2nd volume. / Ed. Mironova A.N. et al. M., Griffith, 2013, 280 p.
6. Bagirova V.L., Krasnyuk I.I., Sboev G.A. Generics: requirements, registration, use // Remedium. 2004. № 3. 34-41p.
7. Validation of analytical techniques for drug manufacturers: a typical guide to an enterprise for manufacturing of medicines. / Ed. Beregovykh V.V. M.: Literra, 2008. 132 p.
8. On the circulation of medicinal products: Federal Law of the Russian Federation dated April 12, 2010 No. 61-FZ.
9. Guidance on the validation of drug analysis techniques (guidelines) / Ed. Yurgelia N.V., et al. M .: “Sport and Culture – 2000”. 2007. 192 p.

ՀԵՂՈՒԿ ՔՐՈՄՈՏՈԳՐԱՖԻԱՅԻ ԿԻՐԱՌՈՒԹՅՈՒՆԸ ՀԻՊՈՏԵՆԶԻՎ ԴԵՂԱՆՅՈՒԹ ԿԱԼԻՈՒՄԻ ԼՈԶԱՐՏԱՆԻ ՈՐԱԿԻ ՎԵՐԱՀՍԿՄԱՆ ՀԱՄԱՐ

Լ.Ա. Օնուչակ, Մ.Բ. Վասիլևա

Սամարայի ակադեմիկոս Ս.Պ. Կորոլյովի անվան հետազոտությունների ազգային համալսարան

Մշակված է կալիումի լոզարտանի հիպոտենզիվ դեղանյութի, վերջինիս սուբստանցիայում նորմալացված խառնուրդի և դեղահաբային (հետադարձ-ֆազային բարձր արդյունավետությամբ հեղուկ քրոմոտոգրաֆիայի կիրառմամբ) տեսքով որոշման մեթոդոլոգիան:

Կալիումի լոզարտանի և խառնուրդի քանակաչափական որոշումն իրականացվել է արտաքին ստանդարտի մեթոդով: Փորձարկման արդյունքում ստացված տվյալները հաստատել են առաջարկված մեթոդիկայի սելեկտիվությունը, ճշգրտությունը և զգայունությունը:

Դեղանյութերի մեջ կալիումի լոզարտանի առկայությունը հնարավոր է նաև հաստատել նրբաշերտ քրոմոտոգրաֆիայի մեթոդով (ելյուենտ՝ ֆոսֆորային բուֆեր (p^H=3,6)-ացետոնիտրիլ,60:40% բաղադրությամբ):

Բանալի բառեր. ընդունիչ, հիպոտենզիվ պատրաստուկ, մետաբոլիտ, քրոմոտոգրաֆիական փորձարկում

ПРИМЕНЕНИЕ ЖИДКОСТНОЙ ХРОМАТОГРАФИИ ДЛЯ КОНТРОЛЯ КАЧЕСТВА ГИПОТЕНЗИВНОГО ЛЕКАРСТВЕННОГО ПРЕПАРАТА «ЛОЗАРТАН КАЛИЯ»

Л.А. Онучак, М.В. Васильева
Самарский национальный исследовательский университет имени академика С.П. Королева

Разработана методика определения гипотензивного лекарственного вещества лозартана калия и его нормируемой примеси в субстанции и в таблетированной форме с применением обращенно-фазовой высокоэффективная жидкостная хроматография (ОФ ВЭЖХ). Количественное определение лозартана калия и примеси проводили методом внешнего стандарта. Экспериментальные данные, полученные при валидации, подтвердили селективность, прецизионность, правильность и чувствительность предложенной методики. Наличие лозартана калия в лекарственных средствах может быть подтверждено также методом обращенно-фазовой тонкослойная хроматография (ТСХ) с применением элюента «фосфатный буфер(pH=3,6) -ацетонитрил» состава 60:40% об.

Ключевые слова: рецептр, гипотензивный препарат, метаболит, храмотографический эксперимент