Calcium channel blockers prevent calcium from entering cells of the heart and blood vessel walls, resulting in lower blood pressure. Calcium channel blockers, also called calcium antagonists, relax and widen blood vessels by affecting the muscle cells in the arterial walls. Physico-chemical analysis methods are increasingly being introduced into fundamental pharmaceutical research and pharmaceutical analysis practice, taking into account their high sensitivity, accuracy, specificity and expressiveness.

Analytical method development is increasingly being introduced into fundamental pharmaceutical research and pharmaceutical analysis practice, taking into account their high sensitivity, accuracy, specificity and expressiveness.

Search criteria was analytical method development for medicines from group of calcium channel blockers. Literature survey has been done in range of years 1990-2018 to make the review updated and comprehensive and to show the new approaches to the development of the methods of analysis of calcium channel blockers. The sources were world recognized journals and key words used as filter were calcium channel blockers, amlodipine, nifedipine, verapamil, validation, method development, spectrophotometry, HPLC, UHPLC. Chromatographic methods of analysis amongst others have the greatest specificity and objectivity and allow for qualitative and quantitative determination of API in combined dosage forms and biological fluids without prior separation of the components. We can conclude that analysts are constantly working on developing new methods of analysis and their optimization in order to save time and consumables, which also ensures the efficiency of the developed method. The main disadvantage of the described methods of API analysis can be considered long term from the beginning of chromatography to API release and specific solvents used as the mobile phase in HPLC. It is necessary to develop methods and to select such chromatographic conditions that will provide high speed and high efficiency at lower pressure of the system. This reduces the amount of used mobile phase, which reduces cost analysis accordingly, while at the same time providing the necessary specificity, accuracy and reproducibility of the results of the analysis during quality control. Also, the reduction of analysis time is achieved by simplifying the conditions for sample preparation.

**Keywords:** Calcium channel blockers, Amlodipine, Nifedipine, Verapamil, Validation

**INTRODUCTION**

Calcium channel blockers prevent calcium from entering cells of the heart and blood vessel walls, resulting in lower blood pressure. Calcium channel blockers, also called calcium antagonists, relax and widen blood vessels by affecting the muscle cells in the arterial walls.

Some calcium channel blockers have the added benefit of slowing heart rate, which can further reduce blood pressure, relieve chest pain (angina) and control an irregular heartbeat.

Some calcium channel blockers are available in short-acting and long-acting forms. Short-acting medications work quickly, but their effects last only a few hours. Long-acting medications are slowly released to provide a longer lasting effect [1].

Calcium channel blockers were first identified in the lab of German pharmacologist Albrecht Fleckenstein beginning in 1964. In 1921, Avicenna introduced the medicinal use of Taxus baccata for phytotherapy in The Canon of Medicine. He named this herbal drug “Zarnab” and used it as a cardiac remedy. This was the first known use of a calcium channel blocker drug, which were not in wide use in the Western world until the 1960s. [2].

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Search criteria was analytical method development for medicines from group of calcium channel blockers. Literature survey has been done in range of years 1990-2018 to make the review updated and comprehensive and to show the new approaches to the development of the methods of analysis calcium channel blockers. The sources were world recognized journals and key words used as filter were calcium channel blockers, amlodipine, nifedipine, verapamil, validation, method development, spectrophotometry, HPLC, UHPLC.

**Methods of analysis of amlodipine**

Amlodipine is a synthetic dihydropyridine and a calcium channel blocker with antihypertensive and antianginal properties. Amlodipine inhibits the influx of extracellular calcium ions into myocardial and peripheral vascular smooth muscle cells, thereby preventing vascular and myocardial contraction. Chemical name of amlodipine is 3-~{O}-ethyl 5-~{O}-methyl 2-[2-aminoethoxyethyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate (fig. 1) [3].

The State Pharmacopoeia of Ukraine (SPBU) does not have a monograph on the substance of amlodipine besylate or on the **Calcium channel blockers**

Calcium channel blockers are first identified in the lab of German pharmacologist Albrecht Fleckenstein beginning in 1964. In 1921, Avicenna introduced the medicinal use of Taxus baccata for phytotherapy in The Canon of Medicine. He named this herbal drug “Zarnab” and used it as a cardiac remedy. This was the first known use of a calcium channel blocker drug, which were not in wide use in the Western world until the 1960s.
prepared medical form. However, the United States Pharmacopoeia regulates the determination of amlodipine besylate in substances and tablets. For identification, UV-spectrophotometry and HPLC/UV are proposed. For quantitative determination of amlodipine besylate in tablets-HPLC/UV, respectively. Chromatographic conditions for the determination of amlodipine besylate, tablets are given in the monograph of the United States Pharmacopoeia, which is used in the chromatographic column of category L1 (fixed phase C18) and mobile phase consisting of three components: buffer solution of pH 3.0 with triethylamine, acetonitrile and methanol. The solvent–mobile phase, mobile phase rate=1 ml/min, detection wavelength=237 nm. The proposed method of the United States Pharmacopoeia requires a long sampling.

The European Pharmacopoeia has a monograph on the substance of amlodipine besylate. Identification of amlodipine besylate of the European Pharmacopoeia regulates the absorption spectroscopy in the infrared region and quantitative determination-HPLC/UV. As a solvent, methanol is used, mobile phase–2.5 g/l of ammonium acetate solution P: methanol P (30:70, V/V), mobile phase rate–1.5 ml/min, detection wavelength–237 nm.

Methods of quantitative determination of amlodipine besylate by the method of spectrophotometry are described in the scientific literature. Products of reaction with various reagents and by their own light absorption, kinetic-spectrophotometric method in substance and drugs, by the method of inhibition of chemiluminescence, by chromatography methods.

Historical development of methods for the quantitative determination of amlodipine besylate in substances and drugs is closely related to the development of analytical methods themselves and pharmaceutical analysis in general. Nowadays the literature contains a large number of scientific papers devoted to the quantitative determination of amlodipine besylate and other APIs in one medical form, since amlodipine is used in combination with various APIs. The HPLC method is widely used in the analysis of amlodipine in medical form, since amlodipine is used in combination with various APIs.

The proposed method uses the chromatographic column Phenomenex C18, 5 μm, 250 x 4.6 mm i.d. column and mobile phase–a mixture of methanol, acetonitrile, water (40:50:10, V/V/V). The method is linear in the range of concentrations of 0.5–60 μg/ml and 0.5–80 μg/ml of enalapril maleate and amlodipine besylate, respectively. The retention time for enalapril maleate and amlodipine besylate was 2.27 and 5.07 min, respectively [28, 29].

Soham S Chitangle et al. developed the method of reverse-phase HPLC with UV-spectrophotometric detection of amlodipine and metoprolol in tablets using Kromasil C18 column (250 x 4.6 mm, 5 μm) and mobile phase–a mixture of 0.02 M phosphorus buffer solution and acetoneitrile (70:30 V/V, pH 3.0), mobile phase rate=1.0 ml/min, detection wavelength=221 nm [31].

The retention time for amlodipine was 2.57 min and metoprolol was 4.49 min. The linearity of the method was determined in the range of concentrations of 10-110 μg/ml of both analytes [32-34]. The same authors proposed the method of reverse-phase HPLC with UV-spectrophotometric detection of the determination of amlodipine and valsartan in capsules using the Kromasil C18 column (250 x 4.6 mm, 5 μm) and mobile phase–a mixture of acetoneitrile and phosphate buffer solution (0.02M, pH 3.0), mobile phase rate=1.0 ml/min, detection wavelength=234 nm. The retention time for amlodipine was 2.02 min and valsartan was 6.20 min. Shi-Ying Dai et al. developed the method of reverse-phase HPLC with UV-spectrophotometric detection of the determination of amlodipine and ramipril in the presence of impurities in the mode of gradient elution [35]. Pradyusha W. et al. proposed the HPLC/UV method for the determination of amlodipine besylate, alixerine hemifumarate and hydrochlorothiazide in drugs using kwantran internal standard. Chromatographic conditions: Inestsil column–ODS C18 (100x4.6 mm, 5 μm), mobile phase–a mixture of 0.1 M ammonia buffer solution and acetoneitrile (65:35 V/V), mobile phase rate=1.0 ml/min, detection wavelength=232 nm. The retention time for amlodipine was 5.22 min, alixerine hemifumarate was 3.90 min, and hydrochlorothiazide was 1.91 min [36-49].

Shalini Pant et al. described the HPLC/UV method for the determination of amlodipine besylate, alikerine hemifumarate and hydrochlorothiazide in drugs using kwantran internal standard. The proposed method uses the chromatographic column Phenomenex C18, 5 μm, 250 x 4.6 mm i.d. column and mobile phase–a mixture of acetoneitrile and phosphate buffer solution (50:50 V/V), mobile phase rate=1.0 ml/min, detection wavelength=230 nm. The retention time for amlodipine was 6.4 min, bisoprolol was 4.4 min. The linearity of the method was studied in the range of concentrations of 1.3-10.8 μg/ml [50].

Reverse-phase HPLC with UV-spectrophotometric detection method for the determination of amlodipine besylate and bisoprolol fumarate in drugs using a C18 column (250 x 4.6 mm, 5 μm) and mobile phase–a mixture of acetoneitrile and phosphate buffer solution (50:50 V/V), mobile phase rate=1.0 ml/min, detection wavelength=230 nm. The retention time for amlodipine was 6.4 min, bisoprolol was 4.4 min. The linearity of the method was studied in the range of concentrations of 2-20 μg/ml and 16-160 μg/ml amlodipine and telmisartan, respectively. The retention time for amlodipine and telmisartan was 3.12 and 5.80 min, respectively [51-55].

Raju et al. described the method of reverse-phase HPLC with UV-spectrophotometric detection for the determination of amlodipine and perindopril in combined medical form using Xten® a C18 column (100 x 4.6 mm, 5 μm) and mobile phase–a mixture of phosphorus buffer solution and acetoneitrile (65:35 V/V), mobile phase rate=0.6 ml/min, detection wavelength=237 nm. Linearity was studied in the range of concentrations of 10-50 μg/ml of amlodipine and 200-1000 μg/ml of perindopril. The retention time for amlodipine and perindopril was 8.51 and 5.28 min, respectively [55-64].

The literature describes bioanalytical methods for the quantitative determination of amlodipine besylate in plasma. Blatt J. et al. developed the method for the determination of amlodipine and amlodipine in blood plasma using HPLC/MS and HPLC/MS/MS. The proposed method uses the chromatographic column Hypersil BDS C18, and internal standard–imipramide. The method is linear in the range of concentrations of 0.1-10 ng/ml. The total time of chromatography was 3.2 min [65].
Alsarra I. A. proposed the bioanalytical method for the determination of amlodipine in blood plasma and medical form using the developed methodology for the study of pharmacokinetics. The method was proposed using C18 Hypersil HyPurity column (3 mmicron, 39 mm id x 150 mm) and mobile phase containing a mixture of acetonitrile, potassium dihydrogen phosphate buffer solution (0.05 M) and acetic acid (62:38:0.1 V/V/V), mobile phase rate–1.8 ml/min. LOD of amlodipine benzyate–1.0 ng/ml, limit of quantitation (LOQ)–10.0 ng/ml. The developed method was used for the analysis of amlodipine tablets and for the study of pharmacokinetics [66].

Feng Y. et al. described the HPLC/MS method for the determination of amlodipine in blood plasma. After extraction with ethyl acetate using the internal standard of nicardin, the chromatographic column C18 and mobile phase–a mixture of methanol and 1% acetic acid solution (65:35 V/V) were used [67].

Zarghi A. et al. developed the bioanalytical method for the determination of amlodipine in blood plasma using the described methodology for the study of pharmacokinetics. The developed procedure uses the chromatographic column Nucleosil C8 (5 μm, 125 mm x 4.6 mm i.d. column and mobile phase–a mixture of acetonitrile and 0.01 M sodium dihydrogenphosphate buffer solution (37:63, V/V), mobile phase rate–1.5 ml/min, detection wavelength–239 nm. Linearity of the method was studied in the concentration range of 0.5–16 ng/ml. LOQ of amlodipine benzyate–0.2 ng/ml [68].

Bahrami G. et al. proposed the express HPLC method for the determination of amlodipine in serum with fluorescence detection and application of the developed method for pharmacokinetic studies. Amlodipine was extracted from the blood serum of ethyl acetate. Pre-colon derivatization with 4-chloro-7-nitrobenzofuran was carried out. Chromatography was performed using the C18 column and mobile phase consisting of phosphate buffer solution pH 2.5 (1 ml/l of triethylamine and methanol), mobile phase rate–2.8 ml/min. As internal standard, propranolol was used. The method of quantitative determination of amlodipine benzyate in blood serum was linear in the range of concentrations of 0.25–16 ng/ml. LOQ was 0.25 ng/ml. The developed method found its application for the study of bioequivalence of tablets of amlodipine 10 ng [69, 70].

Scientists Y. Ma, F. Qin, X. Sun studied the pharmacokinetics of amlodipine using UPHLC/MS. Chromatography was performed using the chromatographic column ACQUITY UPLC BEH C (18) column (50 mm x 2.1 mm, id, 1.7 μmimicro) and gradient elution (mobile phase–water and acetonitrile) with mobile phase rate–0.35 ml/min, internal standard–nimodipine. The method was linear in the range of 0.15–16.0 ng/ml with lower limit of quantitation (LOQ)–0.15 ng/ml. The described method was used to study pharmacokinetics in clinical studies [71, 72].

Scientists Q. Yu, Z. Y. Hu, F. Y. Zhu developed HPLC/MS method for the determination of amlodipine and atorvastatin in blood plasma. The method was proposed using a C18 chromatographic column and gradient elution of mobile phase containing a 0.1% solution of musk acid in water and 0.1% of solution of musk acid in acetonitrile, mobile phase rate–0.4 ml/min. The developed method was used for the determination of amlodipine and atorvastatin in joint application for the treatment of patients with hypertension [73].

Wei X. et al. proposed the bioanalytical method for the determination of amlodipine and nicardin in blood plasma using a monolithic cation exchange column. The developed method was linear in the range of concentrations of 0.5–50.0 ng/ml of both analytes, correlation coefficient–0.998, LOQ–0.2 ng/ml of amlodipine and nicardin [74].

Scientists Ramani A. V., Sengupta P., Mullangi R. developed the UHPLC/MS method for the determination of amlodipine, simvastatin and valsartan in blood plasma with the use of pharmacokinetics. The method was proposed using the chromatographic column X-Terra C18 column and mobile phase containing a mixture of 0.02 M ammonium (pH 4.5) and acetonitrile (20:80 V/V), mobile phase rate–0.5 ml/min. The method is linear in the range of 0.5–50 ng/ml of valsartan and 0.2–50 ng/ml of amlodipine and simvastatin. The correlation coefficient was more than 0.996 for all analytes. The developed method was used for pharmacokinetic studies [75–77].

Methods of analysis of nifedipine

Nifedipine is a dihydropyridine calcium channel blocking agent. Nifedipine inhibits the transmembrane influx of extracellular calcium ions into myocardial and vascular smooth muscle cells, causing dilation of the main coronary and systemic arteries and decreasing myocardial contractility. Chemical name of nifedipine is dimethyl 2,6-dimethyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (fig. 2) [3].

The State Pharmacopoeia of Ukraine (SPuU) has a monograph on the substance of nifedipine. For identification of the substance of nifedipine, the SPuU offers the determination of melting point, absorption spectra in the infrared region and UV-spectrophotometry. For quantitative determination of nifedipine in substances and tablets For identification, the definition of absorption spectrophotometry in the infrared region and UV-spectrophotometry is proposed. For quantitative determination of nifedipine in tablets–HPLC/UV method. In accordance with this article, the following chromatographic conditions are used: chromatographic column of category L1 (with fixed phase C18) with id 4.6 mm x 250 mm; mobile phase–acetonitrile: methanol: water (25:25:50); solvent–methanol, wavelength–235 nm, flow rate–1.0 ml/min.

The European Pharmacopoeia has a monograph on the substance of nifedipine. For identification, it is proposed to determine the melting point, absorption spectrophotometry in the infrared region, TLC (mobile phase–ethyl acetate P and cyclohexane P (40:60) and qualitative reaction to the primary aminogroup–reaction of formation of azo dye (after preliminary reduction of nitro group to amino group). For the quantitative determination of nifedipine–method of cerimetry.

Methods of quantitative determination of nifedipine by methods of spectrophotometry and chromatography are described in the scientific literature.

Scientists Sathis Kumar Dinakaran, Babitha Alluri, Koushik Reddy Annareddy developed the spectrophotometric method for the determination of nifedipine and atorvastatin in substances and medical form. For the analysis of nifedipine and atorvastatin, it is recommended to use the UV-spectrophotometry method in methanol, whose solution has maxima at wavelengths of 237 nm (atorvastatin) and 297 nm (nifedipine) [98].

Revathi R. et al. proposed spectroscopic determination of nifedipine using 40% sodium salicylate solution as hydroscopic solubilizing agent. The solutions of complex have absorption maxima at 350 nm and are subject to the Bouger-Lambert-Beer’s law in the range of concentrations of 2.0–100 μg/ml. The proposed method was successfully applied to determine APIs in tablets [99, 100].

Spectrophotometric determination of nifedipine in drugs, blood serum and urine using oxidation-reduction reactions was developed by scientists Tulassamma P. and Venkateswarlu P. The proposed methods are based on the restoration of the nitro group to the amino group. Method A is based on oxidation followed by the combination of nifedipine and 3-methyl-2-benzothiazolinone hydrazone in the
presence of iron chloride to form a green chromogen color at 685 nm. Method B is based on the oxidation reaction of nifedipine brucine to form a purple chromogen at 546 nm [101].

Modi D. Sita V., Patel Paresh U. developed the method for determination of nifedipine and telmisartan in mixtures. As a solvent, methanol was used. The maximum absorption of nifedipine–235 nm and telmisartan–297 nm. The method was linear in the range of 2-20 μg/ml of nifedipine and 1-10 μg/ml of telmisartan. The developed method can be used in routine analysis of drugs [102].

Scientists AliQamam S. M., AliOyan A. M. proposed the spectrofluorimetric method for the determination of certain substances from the group of 1,4-dihydropyridine, namely, nicardipine, nifedipine, isradipine in drugs and biological fluids. The method is based on the restoration of nicardipine, nifedipine, isradipine from Zn/HCl, and fluorescence measurement at λ_{max}/λ_{em} at 460/364, 450/393 and 446/360 nm, respectively. The method was linear in the range of concentrations 0.4-6.0, 0.2-4.0 and 0.1-9.0 μg/ml with LOD 0.0028, 0.017 and 0.016 μg/ml, respectively. The proposed method was successfully applied for the determination of nicardipine, nifedipine, isradipine in medical form and biologically advanced liquids (plasma, urine) [103].

Scientists Bing L., Hu D. F., Liu F. developed the HPLC/UV method for the determination of nifedipine and atenolol in tablets. According to the proposed method, the following chromatographic conditions are used: chromatographic column Phenomenex -ODS 3 column (250 mm×4.60 mm, 5 μm); mobile phase–methanol: water: phosphate buffer solution pH 7.0 (65:35, V/V), and flow rate–1.0 ml/min. The linear dependence is maintained at intervals of 10-250 μg/ml of atenolol (correlation coefficient 0.9999) and 4-100 μg/ml of nifedipine (correlation coefficient 1.0000) [104].

Scientists Cristobal Galan-Rodriguez, Jaime Gonzalez-Alvarez, Marius Vasile-Remoli proposed the UHPLC/UV for the determination of nifedipine and its impurities. The chromatography column Acquity Shield RPu (50×3.0 mm, 1.8μm) and mobile phase – a mixture of 10 mmol ammonium (pH 4.5) and methanol, were used at flow rate of 0.5 ml/min. The total time of chromatography was 11 min. The method was linear in the range of 0.25-1.5 μg/ml of nifedipine and its impurities. LOQ–0.05 μg/ml [105, 106].

Rosseel M. T. et al. developed the method for determination of nifedipine in blood plasma using capillary gas chromatography with nitrogen detection. Nifedipine was extracted from plasma with toluene. Nitrendipine was used as an internal standard. The method was linear in the range of 2-300 ng/ml [107].

Scientists Soons P. A., Schellens J. H., Rooseman M. C., Breimer D. D. described the analysis of nifedipine and its pyridine metabolite of dihydronifedipine in blood plasma using the method of reverse-phase HPLC/UV. The published method in 1991 was observational and of recommendatory nature [108].

Guo Y. et al. proposed the bioanalytical method for the determination of nifedipine in plasma and its application for the study of bioequivalence. HPLC/MS method for the determination of nifedipine in blood plasma was developed. Nifedipine was extracted with diethyl ether, dimethoxane was used as an internal standard. The chromatography column BDS C (18) and mobile phase–a mixture of methanol and water (66:34, V/V) were used. Researches on the study of bioequivalence were conducted using the developed method of 20 volunteers [109].

Wang D., Jiang K., Yang Sh. developed the bioanalytical method for the determination of nifedipine in blood plasma by the method of UHPLC/MS using the developed methodology for the study of pharmacokinetics. The chromatographic determination was performed using the chromatography column UPLC™ BEH C (18) column and mobile phase–mixture of acetonitrile and 10 mmol/l of ammonium acetate solution (75:25, V/V) at flow rate of 0.2 ml/min. The method was linear in the range of concentrations of 0.104-52.0 ng/ml, LOQ-0.104 ng/ml. The method was fully validated and successfully applied to study the pharmacokinetics of nifedipine tablets [110].

Le Guelec C. et al. described the definition of nifedipine in blood plasma by the method of gas chromatography. The developed method was linear in the concentration range of 0.5-500 ng/ml, LOQ–0.5 ng/ml. The method was used to monitor the concentration of nifedipine in patients receiving this drug [111].

Zendelovska D. et al. proposed the bioanalytical method for determination of nifedipine in blood plasma. Chromatography was carried out using the mobile phase–a mixture of 0.02 mol/l potassium dihydrogen phosphate (pH 4.8) and acetonitrile (42:58, V/V) at wavelength 240 nm. Linearity of method was determined in the range of concentrations 5.0-200.0 ng/ml. LOQ–5.0 ng/ml [112, 113].

Scientists Gurley B. J., Buice R. G., Siddhu P. developed the reverse-phase HPLC/UV method for determination of nifedipine in plasma using an internal standard of 17-α-ethinylestradiol, mobile phase–a mixture of phosphate buffered solution (pH 6.1), methanol, acetonitrile (20:35:45 V/V), chromatography column muBondapak C-18 column at wavelength of 235 nm and flow rate of 1.0 ml/min [114, 115].

Abou-Auda H. S. et al. described the bioanalytical method for the determination of nifedipine in plasma and application of the developed method for the study of pharmacokinetics. Chromatography was performed using a C18 column, mobile phase–a mixture of acetonitrile, methanol and water (35:17:48, V/V) and internal standard–diacetate. The method was linear in the concentration range of 10-200 ng/ml and was successfully applied for pharmacokinetic studies [116].

Scientists Mosab A., Zahaa A., Monir M. proposed the HPLC/UV method for determination of nifedipine in plasma of rats to study pharmacokinetics and bioequivalence. In this method, the chromatographic column Phenomenon Luna-C18 (250 x 4.6 mm, 5 μm) and mobile phase–a mixture of ammonium, methanol, acetonitrile (55: 45: 4, V/V), flow rate–0.8 ml/min, wavelength–235 nm. Linearity of method was determined in the range of concentrations 5.00-200 ng/ml, LOQ–6 ng/ml [117].

Scientists Yritia M., Parra P., Iglesias E., Barbanoj J. M. developed the HPLC/UV method for determination of nifedipine in blood plasma using the chromatographic column C18 and mobile phase–a mixture of acetonitrile and 13 mmol phosphate buffer solution pH 7 (65:35, V/V) at wavelength of 338 nm. LOD–2 ng/ml. The developed method was successfully applied for bioequivalence [118].

Wang X. D. et al. described the HPLC/MS method for the determination of nifedipine and dihydronifedipine in blood plasma, followed by its use for the study of clinical interaction of drugs. Nitrendipine was used as an internal standard. Chromatographic column-Hypersil BDS C (18) column (50 mm x 2.1 mm, i.d., 3 microm) Total chromatography time was 2.5 min. The method was linear in the range of concentrations of 0.5-100 ng/ml of both analytes. LOQ of nifedipine and dihydronifedipine–0.5 ng/ml. The developed method was successfully applied for pharmacokinetic studies [119].

Other scientists also developed the method for determination of nifedipine and dihydronifedipine in blood plasma using the chromatographic column C18 and mobile phase–a mixture of methanol and 50 mmol ammonium acetate solution (50:50, V/V). The method was linear in the range of concentrations of 0.5-100 ng/ml of both analytes [120-121].

Methods of analysis of verapamil

Verapamil is a phenylalkylamine calcium channel blocking agent. Verapamil inhibits the transmembrane influx of extracellular calcium ions into myocardial and vascular smooth muscle and causes dilation of the coronary and systemic arteries and

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decreasing myocardial contractility. Chemical name of verapamil is 2-(3,4-dimethoxyphenyl)-5-[2-(3,4-dimethoxyphenyl)ethyl-methylamino] -2-propan-2-ylpentanenitrile (Rq 3) [3].

The State Pharmacopoeia of Ukraine (SPhU) has a monograph on the substance of verapamil hydrochloride and on verapamil hydrochloride tablets. To identify the substance of verapamil hydrochloride, SPhU offers UV-spectrophotometry, absorption spectrophotometry in the infrared region, TLC (mobile phase-diethylamine P-cyclohexane (15:85)), quantitative determination-alkalimetry, potentiometric titration. For identification of verapamil hydrochloride in tablets, SPhU proposes UV-spectrophotometry, HPLC/UV (mobile phase—a mixture of heptylamine P-acetic acid of ice P-acetone P—solution of l.36 g/l of sodium acetate P (1:4.7:5.81:3.77)), qualitative reaction to chlorides. For quantitative determination of verapamil hydrochloride in tablets UV-spectrophotometry.

The United States Pharmacopoeia regulates the definition of verapamil hydrochloride in substance, tablets and injection solution. In order to identify verapamil hydrochloride in the substance, determination is made by absorption spectrophotometry in the infrared region, HPLC/UV (mobile phase-analogous to SPhU), qualitative reaction to chlorides, for quantitative determination—acidimetry in non-aqueous medium. For the identification of verapamil hydrochloride in tablets, the United States Pharmacopoeia offers the absorption spectrophotometer method in the infrared area and HPLC/UV. The drug Verapamil hydrochloride, tablets are described in the monograph of the United States Pharmacopoeia. According to this article, the HPLC/UV is regulated by the following chromatographic conditions: chromatographic column of category L1 (fixed phase C18) size 4.6 mm x 125 or 150 mm; mobile phase—acetone P: 2-aminoheptane: solution A (0.015 M solution of sodium acetate containing 33 ml/l of acetic acid) in ratio (30:0.5:70); wavelength—278 nm, flow rate—0.9 ml/min.

The European Pharmacopoeia [3] suggests identifying verapamil hydrochloride UV—spectrophotometry sample, absorption spectrophotometry in the infrared region, TLC (mobile phase-diethylamine P-cyclohexane (15:85)) and qualitative reaction to chlorides, quantitative determination—alkalimetry potentiometric titration.

Methods of quantitative determination of verapamil hydrochloride by spectrophotometry, electrochemical method and chromatography methods are described in the scientific literature.

Sahi D. M. proposed spectrophotometric determination of verapamil in drug using bromotymol blue. The method is based on the reaction of complex formation. The reaction product was extracted with tetrachloromethane and the optical density was measured at 420 nm. The developed method was completely validated. Coefficient of correlation—0.9951, LOQ –187.5 μg/ml. The method was successfully applied for the analysis of medical form containing verapamil hydrochloride in tablets UV-spectrophotometry.

Scientists Rahman N., Hoda M. N. developed the spectrophotometric method for the determination of verapamil hydrochloride in drugs using chloramine-T. The method is based on the oxidation of verapamil potassium hydrochloride with metaperiodate in environment of sulphate acid to the formation of colored reaction product having a maximum absorption at 425 nm and subject to the Bouguer-Lambert-Beer’s Law in the range of 12.5–187.5 μg/ml. Method B is based on the formation of colored reaction product with tropaeolin 00 at pH 4.0, which is excreted by chloroform, has a maximum absorption at 400 nm and is subject to the Bouguer-Lambert-Beer’s law in the range of 2.0–30.0 μg/ml. Both methods have found their application in the analysis of drugs containing verapamil hydrochloride [125–130].

Ivanova V. et al. developed the HPLC/UV method for determination of verapamil in blood plasma using the chromatographic column Lichrospher 60 RP-select B column (250 mm × 4 mm ID, 5 μm) and mobile phase—a mixture of 40% acetone and 0.025 mol/l of potassium dihydrogenphosphate pH 2.5, flow rate—1 ml/min, detection wavelength—200 nm. The method was linear in the range of 10–500 ng/ml. The developed method was successfully applied for routine analysis of verapamil in blood plasma [131–133].

Sawicki W. proposed the validated method for determination of verapamil and its metabolite norverapamil in blood plasma. LOD of verapamil is 0.924 μg/ml, LOQ–3.080 μg/ml. LOD of norverapamil is 0.030 ng/ml, LOQ–1.001 ng/ml. The total time of chromatograph making was 8.0 min, the sensitivity was 0.009253 μg/ml. The method was used to monitor the concentration of 200.0 μg/ml. The developed method was successfully applied for carrying out of pharmacokinetic studies and researches on studying bioequivalence of drugs containing verapamil [134, 135].

Scientists Stagni G. and Gillespie W. R. described the method for the determination of verapamil and its norverapamil metabolite in blood plasma. Norverapamil is rapidly acetylated to N-acetylnorverapamil using 2% butanol in hexane. Verapamil and N-acetylnorverapamil are separated using a reversed phase column by mobile phase—a mixture of phosphate buffered solution (0.01 M, pH 6.65) and acetoneitrile. The wavelengths of the fluorescence detector were set at 227 nm for excitation and 308 nm for radiation. The method was linear in the range of concentrations of 3–200 ng/ml of verapamil and 2–100 ng/ml of norverapamil. LOD of verapamil was 3 ng/ml and norverapamil–2 ng/ml. The developed method was used to study pharmacodynamics [136].

Jhee OH et al. developed the bioanalytical method for determining verapamil in plasma of rats using mobile phase A (50 mmol ammonium phosphate, pH 4.5) and mobile phase B (50 mmol ammonium phosphate: acetonitrile, 70:30 v/v). Analysis of verapamil and internal standard of propranolol were performed by direct introduction of plasma into the system and eluted, respectively, 22 min (verapamil) and 12 min (propranolol) at mobile phase A of 0.5 ml/min and mobile phase B of 0.15 ml/min. The method was validated. The correlation coefficient was 0.9997. The method was linear in the range of 0.01 to 250 μg/ml. LOD–0.01 μg/ml, LOQ–0.025 μg/ml. The developed method was successfully used for the study of pharmacokinetics [137, 138].

The method of reverse HPLC/UV determination of verapamil in blood plasma was developed by Muscara M. N. and de-Nucci G. P. and nornorverapamil is used as internal standard. The method was completely validated and used for pharmacokinetic studies [139].

Scientists Lau-Cam C. A. and Piemontese D. proposed the HPLC/UV method for determining verapamil in plasma of rats using dextromethorphan as an internal standard and mobile phase—a mixture of methanol, acetonitrile, triethylamine acetate buffer solution (10:30:60 V/V/V/V) with detection wavelength of 235 nm, flow rate of 1.5 ml/min. The method was linear in the concentration range of 0.5–10 μg/ml (correlation coefficient—0.9999). LOD–0.1 μg/ml. The method was used to monitor the concentration of verapamil in the plasma of rats receiving this drug through various routes of administration [140].

Scientists Hedeland M., Fredriksson E., Lennerstrand H., Bondesson U. described the bioanalytical method for the determination of verapamil enantiomers and their N-dimethylated metabolite in blood plasma using HPLC/MS. The mobile phase—a mixture of 85% aqueous solution of ammonium acetate pH 7.4 and a 15% solution of
acetonitrile, flow rate–0.6 ml/min. The developed method was successfully applied for the study of in vivo intestinal permeability [141-144].

CONCLUSION

From the above-mentioned, we can conclude that analysts are constantly working on developing new methods of analysis and their optimization in order to save time and consumables, which also ensures the efficiency of the developed method. The main disadvantage of the described methods of API analysis can be considered long term from the beginning of chromatography to API release and specific solvents used as the mobile phase in HPLC. In our opinion, it is necessary to develop methods and to select such chromatographic conditions that will provide high speed and high efficiency at lower pressure of the system. This reduces the amount of used mobile phase, which reduces cost analysis accordingly, while at the same time providing the necessary specificity, accuracy and reproducibility of the results of the analysis during quality control. Also, the reduction of analysis time is achieved by simplifying the conditions for sample preparation.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICT OF INTERESTS

Declared none

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