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DEVELOPMENT OF THE METHOD FOR QUANTITIVE DETERMINATION OF AN ACTIVE SUBSTANCE IN "TAMSULOPROST" SUPPOSITORIES

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Key words: suppositories; quantitative determination; high performance liquid chromatography (HPLC); Tamsulosin hydrochloride

Prostatic hyperplasia is one of the most common diseases among elderly men. An integral condition for appearance and development of benign prostatic hyperplasia is the poor state of androgen production in men. An urgent task for the contemporary pharmaceutical science is to create new effective medicines. The prominent place in the treatment of prostate diseases is occupied by alpha-adrenoblockers, which are drugs of the first-line treatment, such as Tamsulosin hydrochloride being a selective and competitive blocker of postsynaptic α_{1A}-adrenergic receptors. Selectivity of Tamsulosin to α_{1A}-adrenergic receptors located in the bladder is several times greater than its ability to interact with α_{1B}-adrenoceptors that are located in the vascular smooth muscles. Therefore, the use of Tamsulosin in the treatment of prostate diseases does not affect the patients blood pressure. The aim of the work was to develop the method for quantitative determination of the active substance Tamsulosin hydrochloride in "Tamsuloprost" suppositories for the treatment of prostatic hyperplasia. Development of the assay method was performed on a Specord 200 spectrophotometer (Analytik Jena, Germany) and a ProStar analytical chromatograph (Varian, USA). The authors have suggested the method for quantitative determination of the active substance Tamsulosin hydrochloride in "Tamsuloprost" suppositories for the treatment of prostatic hyperplasia. During the experiment it has been proven that it is unreasonable to use the method of spectrophotometry in the UV-region to control the content of Tamsulosin in suppositories because of the overlap of two analytical wavelengths of Tamsulosin by the maxima of placebo components. The possibility of using a more specific method – high performance liquid chromatography (HPLC) has been proven and the conditions under which there is a complete separation of placebo components and the active substance within the time taken have been proposed.

Benign prostatic hyperplasia (BPH) is one of the most common urological diseases among men, which leads to a sharp deterioration in the quality of life, urinary disorders, renal dysfunction, erectile dysfunction. The prevalence of this disease increases with age. Thus, the first clinical manifestations of BPH are present in 25% of men at the age of 40 [9].

The task of the prostatic hyperplasia pharmacotherapy is to reduce the severity of symptoms, to lower the risk of acute urinary retention, and therefore, to reduce the need to perform surgery and improve the overall quality of life of patients [3, 4].

At present the main therapeutic method for patients with clinical manifestations of BPH is the use of α_1-adrenoblockers. Administration of α_1-blockers reduces pathological symptoms and improves the urinary flow rate due to relaxation of smooth muscles of the prostate and the bladder neck. Tamsulosin hydrochloride is a selective and competitive blocker of postsynaptic α_{1A}-adrenergic receptors located in the smooth muscles of the prostate, the bladder neck and the prostatic part of the urethra. Due to the high selectivity to α_{1A}-adrenergic receptors Tamsulosin has no effect on blood pressure of patients [1, 2].

Today, the most appropriate dosage form for the treatment of prostate hyperplasia is a suppository. At the pharmaceutical market there are no medicines with α-blockers in the form of suppositories, so their development and research is an important and promising direction for the modern pharmaceutical science.

For the further drug quality assurance it is necessary to develop analytical approaches that will ensure comprehensive monitoring for compliance with the existing requirements. Development of the method for the quantitative determination of active pharmaceutical ingredients (API) in a drug is an important and necessary step when preparing the Quality Control Methods project. For the Tamsulosin assay different authors proposed spectrophotometric [8, 10] and chromatographic [5, 6, 7] methods. Considering the complex matrix of the medicine it was necessary to identify and work out the most appropriate method to use.

The aim of the work was to develop the method for quantitative determination of the active substance Tamsulosin hydrochloride in "Tamsuloprost" suppositories for the treatment of prostatic hyperplasia.

Materials and Methods

The objects of the study were samples of suppositories with the active substance Tamsulosin hydrochloride with the weight of 1.6 g made of a solid fat by moulding. In order to optimize the composition of suppositories

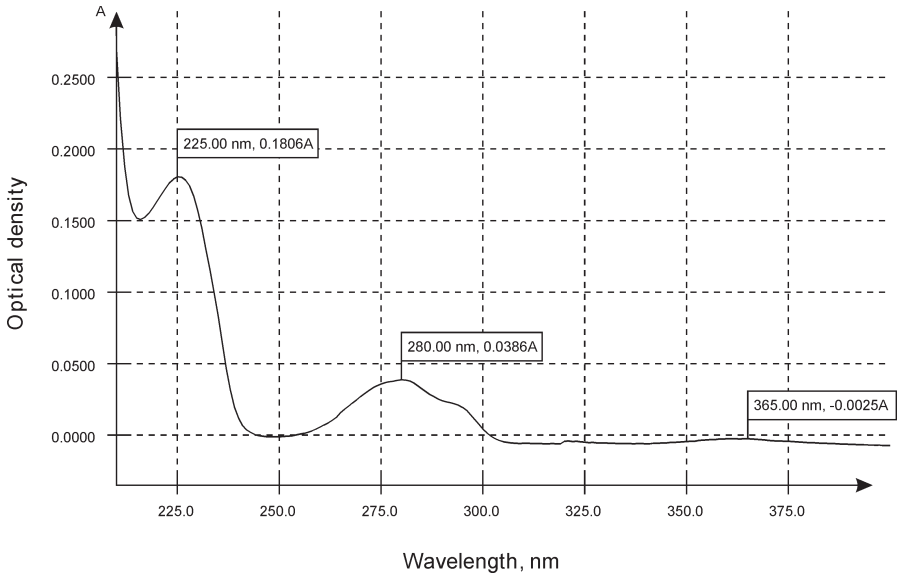

Fig. 1. The absorption spectrum of Tamsulosin (≈ 0.006 mg/ml).

a Lanette SX emulsifier in the amount of 5% was introduced into the base.

Development of the assay method was performed on a Specord 200 spectrophotometer (Analytik Jena, Germany) and a ProStar analytical chromatograph (Varian, USA) using Waters XTerra™ MS C8 columns with 50 mm in length, the diameter of 4.6 mm and the particle size of 2.5 microns with a precolumn.

In the work the following samples of the components of placebo and substances were used: Tamsulosin hydrochloride (Ra Chem Pharma Ltd, s. TAM/021/11/10); Lanette SX emulsifier (Cognis, Germany), solid fat ("Sasol", Germany).

Results and Discussion

To determine the possibility of using the spectrophotometric method in the UV-region compared to the standard solution or by the absorption specific indicator to monitor the content of Tamsulosin hydrochloride in the medicine, the molecular absorption spectra of solutions of Tamsulosin, the suppository base, placebo and the emulsifier were obtained.

As it can be seen from the spectrum (Fig. 1) for Tamsulosin hydrochloride, the analytical wavelength of either 225 nm or 280 nm can be used. However, both analytical wavelengths are covered by peaks of the placebo component, namely of the emulsifier Lanette-SX, proven during the sample preparation, i.e. it is extracted together with Tamsulosin.

Fig. 2a shows the spectra of Tamsulosin hydrochloride and the emulsifier in concentrations of about 0.015 mg/ml and 2.8 mg/ml, respectively (the ratio is 1:186, in the medicine it is 1:200). Thus, at the wavelength of 225 nm the emulsifier will almost twice overstate the results of the quantitative determination of Tamsulosin. Fig. 2b shows the spectra of Tamsulosin Hydrochloride and the emulsifier in concentrations of about 0.015 mg/ml and 0.28 mg/ml, respectively (the ratio is 1:18). So, at the wavelength of 280 nm the emulsifier will overstate the results of the quantitative determination of Tamsulosin by about 20%.

Thus, the use of the spectrophotometric method in the UV-region to determine Tamsulosin hydrochloride in suppositories is difficult due to the significant optical absorption of placebo components. Therefore, to solve this problem a more specific method has been used, namely high performance liquid chromatography (HPLC), and the conditions under which there is a complete separation of the placebo components and active ingredient within the particular time have been proposed. Since the placebo components and Tamsulosin have significant differences in the chemical structure, a different chromatographic behaviour is expected. Taking this into account even a short chromatographic column sharing ability would be enough for an acceptable separation. The use of a short chromatographic column, in its turn, allows to reduce the analysis time and the solvent consumption for chromatography.

Determination was performed on a Waters XTerra™ MS C8 column, 2.5 microns, 4.6×50 mm. When developing the method the various combinations of the mobile phase composition and pH medium were tested. Finally, the method using phosphoric acid – acetonitrile – water in the ratio of 2:32:68 as a mobile phase was chosen; it was also used as a solvent for extraction of Tamsulosin from the dosage form. Under these conditions there was separation of the placebo components and Tamsulosin, the last was eluted with the retention time of about 1.5 min (RF was about 2.0). The analysis time did not exceed 5 minutes. The chromatogram of the test solution is shown in Fig. 3.

On the basis of the research conducted the assay method for Tamsulosin hydrochloride suppositories "Tamsuloprost" was developed.

The tests were performed by the method of liquid chromatography according to the requirements of the SPhU (2.2.29). The solutions were used freshly prepared and protected from light.

Test solution. Place aproximately 3.2 g of the homogenized suppository mass in a 100 ml separation funnel, dissolve it in 50 ml of hexane, then add 7.5 ml of the

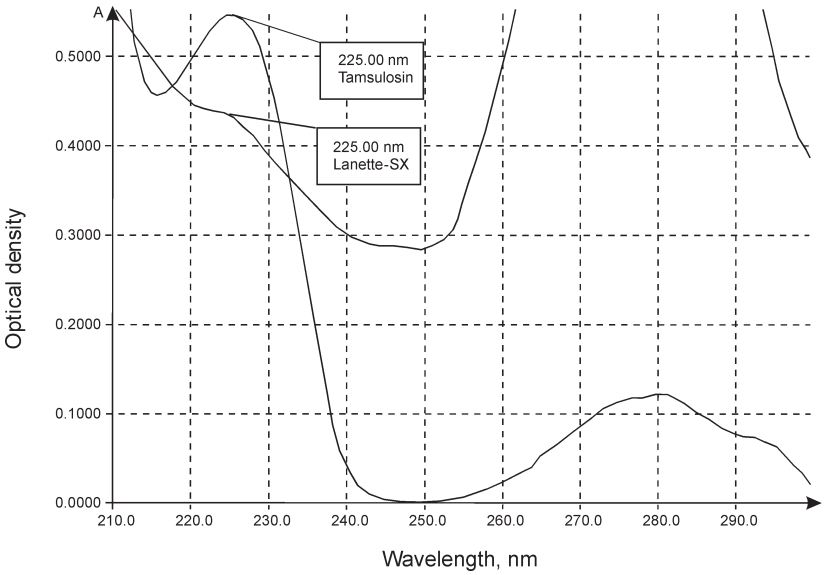

Fig. 2a. Absorption spectra of the emulsifier and Tamsulosin.

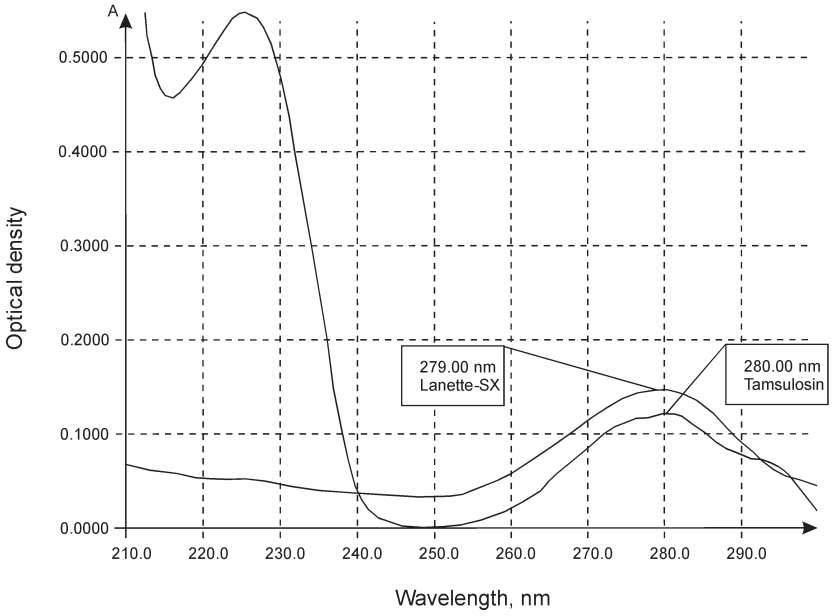

Fig. 2b. Absorption spectra of the emulsifier and Tamsulosin.

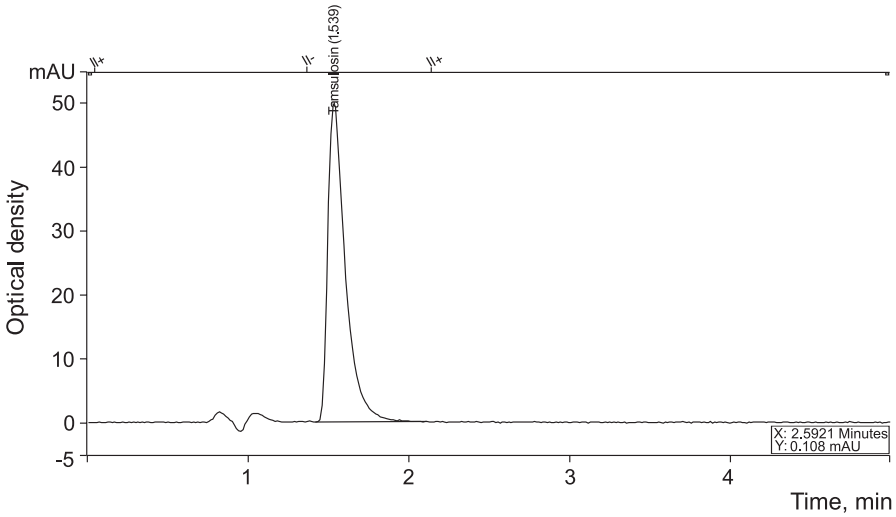

Fig. 3. The chromatogram of the test solution.

mobile phase. Shake the emulsion intensely for 5 minutes and leave for 20 minutes for complete separation of the layers. Collect the bottom layer carefully in a 25 ml volumetric flask avoiding contact with the top layer in the flask. Repeat the extraction twice more using 7.5 ml of the mobile phase. Dilute the flask content to the volume with the solvent for tests and mix.

Blank solution. Place aproximately 0.080 g (accurate weight) of Tamsulosin hydrochloride in a 100 ml volumetric flask, then add 50 ml of methanol, dissolve, dilute to the volume with the same solvent and mix. Place 2 ml of the solution was obtained in a 50 ml volumetric flask, dilute to the volume with the mobile phase and mix.

Before chromatography the solution was filtered through a nylon membrane filter with a pore size not more than 0.45 microns.

Chromatographic conditions were:

– a stainless steel column of 50 mm×4.6 mm filled with octylsilyl silica gel for chromatography, with a particle size of 2.5 microns with a precolumn of 20 mm × 4.6 mm, for example, Waters XTerra™ MS C8 2.5 um 4.6 × 50 mm, or a similar one, for which the requirements of the system suitability test were met;

– the mobile phase rate – 1.0 ml/min;

– detection: spectrophotometry at the wavelength of 280 nm;

– the column temperature – 30°C;

– the sample volume: 20 mcl;

– the mobile phase: a mixture of phosphoric acid – acetonitrile for chromatography – water for chromatography in the ratio of 2:32:68 degassed by any convenient method.

The blank solution was chromatographed for several times. The chromatographic system is considered to be applicable if in the comparison solution chromatogram:

– the efficiency of the chromatographic system calculated by the Tamsulosin peak is not less than 1000 theoretical plates;

– the peak symmetry of Tamsulosin is not more than 2.0;

– the relative standard deviation (RSD) of the Tamsulosin peak area for five chromatograms does not exceed 1.0%.

The test solution was chromatographed at least 3 times.

The content of Tamsulosin hydrochloride (X) in 1 suppository, mg, was calculated using the formula:

$$X = \frac{S_1 \cdot m_0 \cdot 2 \cdot 25 \cdot P \cdot a \cdot 1000}{S_0 \cdot m_1 \cdot 100 \cdot 50 \cdot 100} =$$

$$= \frac{S_1 \cdot m_0 \cdot P \cdot a}{S_0 \cdot m_1 \cdot 10},$$

where: S_1 – is the average value of Tamsulosin peak areas calculated from the test solution chromatograms; S_0 – is the average value of Tamsulosin peak areas calculated from the sum blank solution; m_0 – is the standard sample weight of Tamsulosin hydrochloride used to prepare the sum blank solution, g; m_1 – is the sample weight of the medicine, g; a – is the average weight of a suppository, g; P – is the Tamsulosin hydrochloride content in the standard sample of Tamsulosin hydrochloride, %.

The content of $C_{20}H_{28}N_2O_5S{\cdot}HCl$ (Tamsulosin hydrochloride) in 1 suppository should be from 0.36 mg to 0.44 mg.

Notes: ***Preparation of the mobile phase.*** *Mix 320 ml of acetonitrile for chromatography and 680 ml of water for chromatography, add 2 ml of phosphoric acid. Mix thoroughly and filter through a nylon membrane filter with a pore size not more than 0.45 microns.*

CONCLUSIONS

1. The quantitative determination of the active substance Tamsulosin hydrochloride in "Tamsuloprost" suppositories for the treatment of prostatic hyperplasia has been suggested.

2. It has been proven that it is unreasonable to use the method of spectrophotometry in the UV-region to control the content of Tamsulosin in suppositories because of the overlap of two analytical wavelengths of Tamsulosin by the maxima of placebo components.

3. The possibility to use the HPLC method for assay of Tamsulosin hydrochloride in "Tamsuloprost" suppositories has been substantiated.

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РОЗРОБКА МЕТОДИКИ КІЛЬКІСНОГО ВИЗНАЧЕННЯ ДІЮЧОЇ РЕЧОВИНИ В СУПОЗИТОРІЯХ «ТАМСУЛОПРОСТ»

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Ключові слова: *супозиторії; кількісне визначення; високоефективна рідинна хроматографія (ВЕРХ); тамсулозину гідрохлорид*

Гіперплазія передміхурової залози є одним з найбільш поширених захворювань у чоловіків похилого віку. Неодмінною умовою виникнення і розвитку доброякісної гіперплазії є незадовільний стан продукції андрогенів у чоловіків. Актуальним завданням сучасної фармацевтичної науки є створення нових ефективних препаратів. Чинне місце в терапії захворювань передміхурової залози посідають α-адреноблокатори – препарати першої лінії лікування, тамсулозину гідрохлорид – вибірковий і конкурентний блокатор постсинаптичних α_{1A}-адренорецепторів. Селективність тамсулозину до α_{1A}-адренорецепторів, розташованих у сечовому міхурі, у декілька разів перевищує його здатність взаємодіяти з α_{1B}-адренорецепторами, які знаходяться у гладких м'язах судин. Тому застосування тамсулозину в терапії передміхурової залози не впливає на артеріальний тиск пацієнтів. Метою роботи стала розробка методики кількісного визначення діючої речовини тамсулозину гідрохлориду у супозиторіях «Тамсулопрост» для лікування гіперплазії передміхурової залози. Розробку методики визначення проводили на спектрофотометрі Specord 200 (Analytik Jena, Німеччина) та аналітичному хроматографі ProStar (Varian, США). Авторами статті запропоновано методику кількісного визначення діючої речовини тамсулозину гідрохлориду у супозиторіях «Тамсулопрост» для лікування гіперплазії передміхурової залози. В ході експерименту обгрунтовано недоцільність використання методу спектрофотометрії в УФ-області для контролю вмісту тамсулозину в супозиторіях внаслідок перекривання двох аналітичних довжин хвиль тамсулозину максимумами компонентів плацебо. Доведена можливість використання більш специфічного методу – високоефективної рідинної хроматографії (ВЕРХ) і запропоновані умови, за яких відбувається повне розділення компонентів плацебо та діючої речовини за прийнятний час.

РАЗРАБОТКА МЕТОДИКИ КОЛИЧЕСТВЕННОГО ОПРЕДЕЛЕНИЯ ДЕЙСТВУЮЩЕГО ВЕЩЕСТВА В СУППОЗИТОРИЯХ «ТАМСУЛОПРОСТ»

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Ключевые слова: *суппозитории; количественное определение; высокоэффективная жидкостная хроматография (ВЭЖХ); тамсулозина гидрохлорид*

Гиперплазия предстательной железы является одним из наиболее распространенных заболеваний у мужчин пожилого возраста. Неотъемлемым условием возникновения и развития доброкачественной гиперплазии считается неудовлетворительное состояние продукции андрогенов у мужчин. Актуальным заданием современной фармацевтической науки является создание новых эффективных препаратов. Ведущее место в терапии заболеваний предстательной железы занимают α-адреноблокаторы – препараты первого ряда лечения, тамсулозина гидрохлорид – избирательный и конкурентный блокатор постсинаптических α_{1A}-адренорецепторов. Селективность тамсулозина к α_{1A}-адренорецепторам, расположенным в мочевом пузыре, в несколько раз превышает его способность взаимодействовать с α_{1B}-адренорецепторами, которые находятся в гладких мышцах сосудов. Поэтому применение тамсулозина в терапии предстательной железы не влияет на артериальное давление пациентов. Целью работы стала разработка методики количественного определения действующего вещества тамсулозина гидрохлорида в суппозиториях «Тамсулопрост» для лечения гиперплазии предстательной железы. Разработку методики определения проводили на спектрофотометре Specord 200 (Analytik Jena, Германия) и аналитическом хроматографе ProStar (Varian, США). Авторами статьи предложена методика количественного определения действующего вещества тамсулозина гидрохлорида в суппозиториях «Тамсулопрост» для лечения гиперплазии предстательной железы. В ходе эксперимента обоснована нецелесообразность использования метода спектрофотометрии в УФ-области для контроля содержания тамсулозина в суппозиториях в результате перекрывания двух аналитических длин волн тамсулозина максимумами компонентов плацебо. Приведена возможность использования более специфического метода – высокоэффективной жидкостной хроматографии (ВЭЖХ) и предложены условия, при которых происходит полное разделение компонентов плацебо и действующего вещества за приемлемое время.