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QUALITY CONTROL MEASUREMENT AND *IN VITRO* BIOEQUIVALENCE OF VALSARTAN AND ATENOLOL TABLETS MARKETED IN UKRAINE

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Background. *The urgent issue of hypertension is determined by its high population incidence, significant burden of the disease, risk of disability and impact on life expectancy. Rational combinations of drugs of different pharmacological groups in case of ineffectiveness of monotherapy to achieve the clinical effect of pharmacotherapy are clearly recommended in the world and national recommendations for diagnosis and treatment of hypertension. Therefore, innovative pharmaceutical development of a combination of antihypertensive drugs and creation of domestic drugs with antihypertensive action is an urgent task of contemporary pharmacy.*

Objective. *The aim of this study was to perform the quality control measurements and evaluation of dissolution tests for different brands of valsartan and atenolol tablets available in Ukraine.*

Methods. *The concentrations of valsartan and atenolol in samples (drug content and dissolution study) were determined by the proposed HPLC method.*

Results. *The results of the tests conducted for evaluation of the tablets were found to be in acceptable limits for all the selected brands. The correlation coefficient (R^2) was 0.9991 and the regression equation was y=61.39x+0.3117. It has been established that the equivalence of dissolution profiles for all recommended dissolution media is observed (pH 1.2, 4.5, and 6.8) for the studied drugs. In all three dissolution media, the release rates of valsartan and atenolol of all dosage forms are more than 85% in 15 min. The dissolution profile of all the selected brands was within the standard limits and was acceptable.*

Conclusions. *Analytical method development is an integral part of the quality control measurements and evaluation of dissolution tests. Our previously developed HPLC method is essential for quality control of a large number of samples in short time intervals. Therefore, the method developed by our group is suitable for a routine quality control analysis of any pharmaceutical preparation containing two tested drugs with the suggested chromatographic method advantages for checking quality during dissolution studies of their dosage forms.*

KEYWORDS: **hypertension; valsartan; atenolol; high-performance liquid chromatography; *in vitro* bioequivalence; dissolution.**

Introduction

Valsartan (Fig. 1) is chemically described as (2*S*)-3-methyl-2-[pentanoyl-[[4-[2-(2*H*-tetrazol-5-yl)phenyl]phenyl]methyl]amino]butanoic acid. Valsartan is an orally active nonpeptide triazole-derived antagonist of angiotensin (AT) II with antihypertensive properties. Valsartan selectively and competitively blocks the binding of angiotensin II to the AT1 subtype receptor in vascular smooth muscle and the adrenal gland, preventing AT II-mediated vasoconstriction, aldosterone synthesis and secretion, and renal reabsorption of sodium, and results in vasodilation, increased excretion of sodium and water, reduction of plasma volume, and reduction of blood pressure. Therefore, analytical methods for their separation and quantification in pharmaceutical formulations and inhuman plasma are needed for quality control and therapeutic drug monitoring, respectively. Several techniques have been reported in the literature for determination of valsartan individually and combination with other drug other than atenolol [1-16] in pharmaceutical dosage forms or human serum samples.

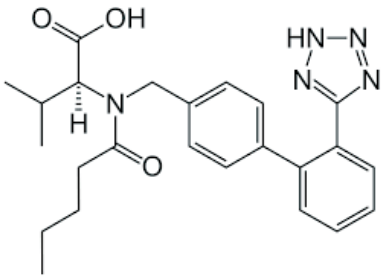

Fig. 1. Chemical structure of valsartan.

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Atenolol (Fig. 2) is a synthetic isopropylamino-propanol derivative used as an antihypertensive, hypotensive and antiarrhythmic. Atenolol is chemically known as 2-[4-[2-hydroxy-3-(propan-2-ylamino)propoxy]phenyl]acetamide. Atenolol acts as a peripheral, cardioselective beta blocker specific for beta-1 adrenergic receptors, without intrinsic sympathomimetic effects. It reduces exercise heart rates and delays atrio-ventricular conduction, with overall oxygen requirements decrease. Numerous analytical methods were reported [17-27] for determination of atenolol in bulk and combination with other drugs other than valsartan.

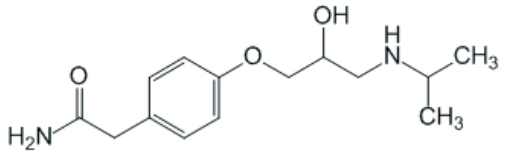

Fig. 2. Chemical structure of atenolol.

The urgent issue of hypertension is determined by its high population incidence, significant burden of the disease, risk of disability and impact on life expectancy. Rational combinations of drugs of different pharmacological groups in case of ineffectiveness of monotherapy to achieve the clinical effect of pharmacotherapy are clearly recommended in the world and national recommendations for diagnosis and treatment of hypertension. Therefore, innovative pharmaceutical development of a combination of antihypertensive drugs and creation of domestic drugs with antihypertensive action is an urgent task of contemporary pharmacy. In the last few decades, the cost of medications is increasing a lot and it is quite challenging to afford lifelong medications for hypertension treatment. Different strategies have been planned by the healthcare systems to reduce the medication costs, improve treatment efficacy and safety and patient compliance with pharmacotherapy [28]. The results of quality control testing, such as friability, weight variation, hardness, percentage purity, and disintegration, suggests the level up to which the GMP guidelines had been followed during the manufacturing of these generic products. When the generic and the innovator brand would have comparable dissolution profile then the *in vivo* bioequivalence test of the generics can be waived. It was reported that fewer generics in the market were counterfeit and of inferior quality than the innovators. So, identifying these fake and suspicious generics is the prime challenge to our health department and quality control units. This research assists highlighting the pharmaceutical products which are found to be spurious, of inferior quality and dangerous to the users.

The objective of the research was to perform the quality control measurements and evaluate dissolution tests of different brands of valsartan and atenolol tablets available in Ukraine.

Methods

Valsartan (purity 99.9%) was purchased from Jubilant Generics Limited (India); atenolol (purity 98.9%) was purchased from Sigma-Aldrich (Switzerland). The methanol and acetonitrile used in experiments was HPLC gradient grade and ammonium acetate and tetramethylammonium hydroxide were of Ph.Eur.reagent grade and was purchased from Merck Darmstdat, Germany. Analytical Balance Mettler Toledo MPC227, pH–metter Metrohm 827, demineralized water by TKA Micro system, with final conductivity less than 0.05μS/cm, were used. IKA orbital shaker KS4000i was used for sample agitation. The nylon and regenerated cellulose RC 0.45um syringe filters were purchased from Agilent Technologies.

Dionex Ultimate 3000 UHPLC system controlled by Chromeleon version 6,80, composed of quaternary LPG pump ultimate 3000, autosampler ultimate 3000, ultimate 3000 column compartment, four channel UV-Vis detector ultimate 3000 RS. Shimadzu Nexera XR UPLC system with LPG Quaternary Pump LC-20AD with degasser DGU-20A5R, Autosempler SIL-20AC, PDA detector M20-A, Column Oven and Controller CBM-20A controlled by Lab Solutions version 5,97. The column Discovery C18 (4.6 mm i.d.×150 mm, 5 μm), purchased from Sigma-Aldrich Supelco, was used.

Sample preparation

Twelve tablets of each preparation were studied to obtain statistically significant results. The tablets of different pharmaceutical manufacters with declared contents of 80 mg valsartan and 100 mg of atenolol were purchased from local drug store, pharmacy. The tablets were put in 100 mL measuring flasks and dissolved in 50 mL 50% v/v methanol, ultrasound crushed and treated for 2 minutes and shake 15 minutes with orbital shaker. After that measuring flasks were filled to mark of 100 mL, the final concentrations were 1mg/mL for atenolol and 0.8 mg/mL for valsartan. The samples

were filtered with RC 0.45um syringe filters and injected.

In vitro dissolution of twelve tablets containing valsartan and atenolol was performed using buffer solutions (pH 1.2; 4.5; 6.8) as the dissolution media at 50 rpm by the USP Apparatus II. The dissolution was studied in a 900 mL volume of buffer solution at 37 °C (±0.5) using the paddle method. One mL of sample was withdrawn and replaced with fresh dissolution medium at the time intervals of 5, 15, 30, 45 minutes [29-32].

The concentrations of valsartan and atenolol in the samples (drug content and dissolution investigation) were determined by the suggested HPLC method.

Results

Previously, we have made method development of valsartan and atenolol in dosage forms. The optimum mobile phase composition was composed of 20% acetonitrile, 80% of 0.16% ammonium acetate and 0.2% of 1.5 M tetramethylammonium hydroxide (V/V), pumped with 1.0 mL/min at 30 °C set temperature of column oven, with UV detector set to 225 nm and 237 nm wavelength. Analyses were performed by means of the column Discovery C18 (4.6 mm i.d.×150 mm, 5 µm) (Fig. 3).

The results of percentage purity of all the brands are shown in Table 1. The drug content was determined to be highest for Valsartan-1 and Atenolol-4. The drug content was assessed once and compared with the calibration curve. The correlation coefficient (R^2) was 0.9991 and the regression equation was y = 61.39x+0.3117.

Table 1. Drug content of different brands of valsartan and atenolol tablets

Brand code	Drug content (%) n=20
Valsartan-1 (Innovator)	99.82±2.24
Valsartan-2	98.12±3.65
Valsartan-3	99.11±2.74
Valsartan-4	98.01±3.64
Atenolol-1 (Innovator)	99.11±1.89
Atenolol-2	97.62±3.67
Atenolol-3	98.55±1.95
Atenolol-4	99.92±2.73

Dissolution test is used to determine the quality of the drug. Comparative dissolution kinetics test is used at all stages of the drugs life cycle. In the development of dosage form for comparative dissolution kinetics test allows assessing the technological correctness of techniques, and thereby increase the probability of positive results for future studies of bioequivalence. In addition to routine quality control tests, comparative dissolution tests are used to waive bioequivalence requirements (biowaivers) for lower strengths of a dosage form. Dissolution study is an important parameter used to predict the bioavailability and *in vivo* drug release performance. Dissolution study is very significant in determining the release of drug from different dosage forms including tablets. The active absorption of oral dosage forms depend on adequate release of drug. Comparative dissolution profiles are shown in Table 2.

The point of 15 min is critical and decisive. Medicine is considered very quick soluble when

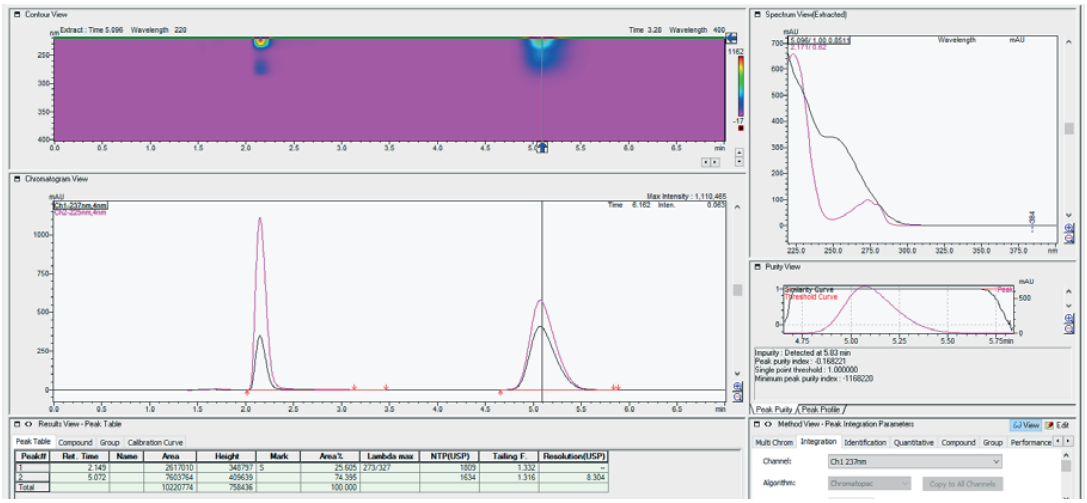

Fig. 3. Chromatogram obtained using Shimadzu Nexera XR UPLC system and mobile phase 20% acetonitrile, 80% of 0.16% ammonium acetate and 0.2% of 1.5 M tetramethylammonium hydroxide (V/V), column Discovery C18 (4.6 mm i.d.×150 mm, 5 µm) at 2 wavelengths 225 nm and 237 nm.

Table 2. Comparative dissolution data of valsartan and atenolol in selected brands

Brand code	Medium	% dissolved 15 min	% dissolved 30 min
Valsartan-1	pH 1.2	95.18	94.59
	pH 4.5	93.84	93.02
	pH 6.8	92.43	93.67
Valsartan-2	pH 1.2	89.98	87.37
	pH 4.5	87.38	89.84
	pH 6.8	85.19	86.38
Valsartan-3	pH 1.2	85.28	86.58
	pH 4.5	87.68	90.01
	pH 6.8	86.84	88.67
Valsartan-4	pH 1.2	85.89	85.98
	pH 4.5	85.96	85.78
	pH 6.8	86.94	86.05
Atenolol-1	pH 1.2	93.96	95.95
	pH 4.5	92.56	93.36
	pH 6.8	90.93	92.58
Atenolol-2	pH 1.2	91.94	92.67
	pH 4.5	89.49	93.06
	pH 6.8	87.56	90.94
Atenolol-3	pH 1.2	91.82	93.37
	pH 4.5	90.01	91.65
	pH 6.8	86.05	89.95
Atenolol-4	pH 1.2	95.96	96.03
	pH 4.5	92.94	94.56
	pH 6.8	91.46	93.14

at least 85% of the active substance dissolves in 15 minutes, quickly soluble – when at least 85% of the active substance dissolves in 30 minutes. According to the obtained data, the equivalence of dissolution profiles for all recommended dissolution media has been established (pH 1.2, 4.5, and 6.8) for the studied drugs. In all three dissolution media, the releases of valsartan and atenolol of all dosage forms were more than 85% in 15 min (Table 2). The dissolution profile of all the selected brands was within the standard limits and was acceptable.

Conclusions

Analytical method development is an integral part of the quality control measurements and evaluation of dissolution test. Our previously developed HPLC method was essential for quality control of a large number of samples in short time intervals. Unavailability and price of innovator brand urges patients to go for alternate options including generic brands. The selected brands were evaluated and compared with those of reference or innovator brand to assure the potential for cure of the disease. Moreover, the results of dissolution studies of all the brands were within the standard limits. This suggested that the proper GMP guidelines were followed during the manufacturing of these brands that proved a good quality. Hence, these generics may be considered to be a substitute for innovator brand in case of unavailability. Thus, all the brands selected for the study complied with the standard specifications and the definite observations on similar efficacy of these generics may be obtained after performing the *in vivo* studies. Therefore, the method developed by our group is suitable for the routine quality control analysis of any pharmaceutical preparation containing two tested drugs with the suggested chromatographic method with advantages for checking quality during dissolution studies of their pharmaceutical preparations.

Conflict of Interests

Authors declare no conflict of interest.

Funding

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ВИЗНАЧЕННЯ КОНТРОЛЮ ЯКОСТІ ТА *IN VITRO* БІОЕКВІВАЛЕНТНОСТІ ТАБЛЕТОК ВАЛСАРТАНУ ТА АТЕНОЛОЛУ РИНКУ УКРАЇНИ

К. Пелешок
ТЕРНОПІЛЬСЬКИЙ НАЦІОНАЛЬНИЙ МЕДИЧНИЙ УНІВЕРСИТЕТ ІМЕНІ І. Я. ГОРБАЧЕВСЬКОГО, ТЕРНОПІЛЬ, УКРАЇНА

Вступ. *Актуальність проблеми артеріальної гіпертензії визначається її високою популяційною частотою, значним тягарем хвороби, ризиком інвалідизації та впливом на тривалість життя людини. У світових і вітчизняних рекомендаціях з діагностики та лікування артеріальної гіпертензії чітко рекомендовано раціональні комбінації препаратів різних фармакологічних груп при неефективності монотерапії для досягнення клінічного ефекту фармакотерапії. Тому, інноваційна фармацевтична розробка комбінації антигіпертензивних засобів та створення вітчизняних лікарських засобів антигіпертензивної дії є актуальним завданням сучасної фармації.*

Мета. *Здійснити контроль якості та оцінити тест розчинення різних марок таблеток валсартану та атенололу, які є представленими на ринку України.*

Методи. *Концентрації валсартану та атенололу у зразках (вміст в лікарських формах та тест розчинення) визначалися запропонованим методом ВЕРХ.*

Результати. *Результати випробувань, проведених для кількісного визначення визначення АФІ таблеток, є прийнятними для всіх обраних марок. Було встановлено, що коефіцієнт кореляції (R^2) становить 0.9991 та рівняння регресії y=61.39x+0.3117. Доведено, що для досліджуваних препаратів спостерігається еквівалентність профілів розчинення для всіх рекомендованих середовищ розчинення (pH 1.2, 4.5 та 6.8). У всіх трьох середовищах розчинення вивільнення валсартану та атенололу всіх лікарських форм перевищують 85% за 15 хв. Оцінка розчинення всіх вибраних марок в межах стандартних лімітів та є прийнятною.*

Висновки. *Розробка аналітичної методики є невід'ємною частиною контролю якості та оцінки тесту розчинення. Розроблена нами методика ВЕРХ є важливою для контролю якості великої кількості зразків за короткі проміжки часу. Тому метод розроблений нашою групою, підходить для рутинного аналізу якості будь-якого лікарського препарату, що містить два випробувані АФІ із запропонованими перевагами хроматографічного методу для перевірки якості під час досліджень розчинення їх лікарських форм.*

КЛЮЧОВІ СЛОВА: **гіпертензія; валсартан; атенолол; високо ефективна рідинна хроматографія; *in vitro* біоеквівалентність; розчинення**

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