# МЕДИЦИНСКИ УНИВЕРСИТЕТ – ПЛЕВЕН

## ФАКУЛТЕТ „ФАКУЛТЕТ ФАРМАЦИЯ“

### ЦЕНТЪР ЗА ДИСТАНЦИОННО ОБУЧЕНИЕ


**Лекция №03**

# Анализ на лекарства, действащи на сърдечно-съдовата система - АСЕ инхибитори, АТ1 антагонисти и вазодилататори, 3 част


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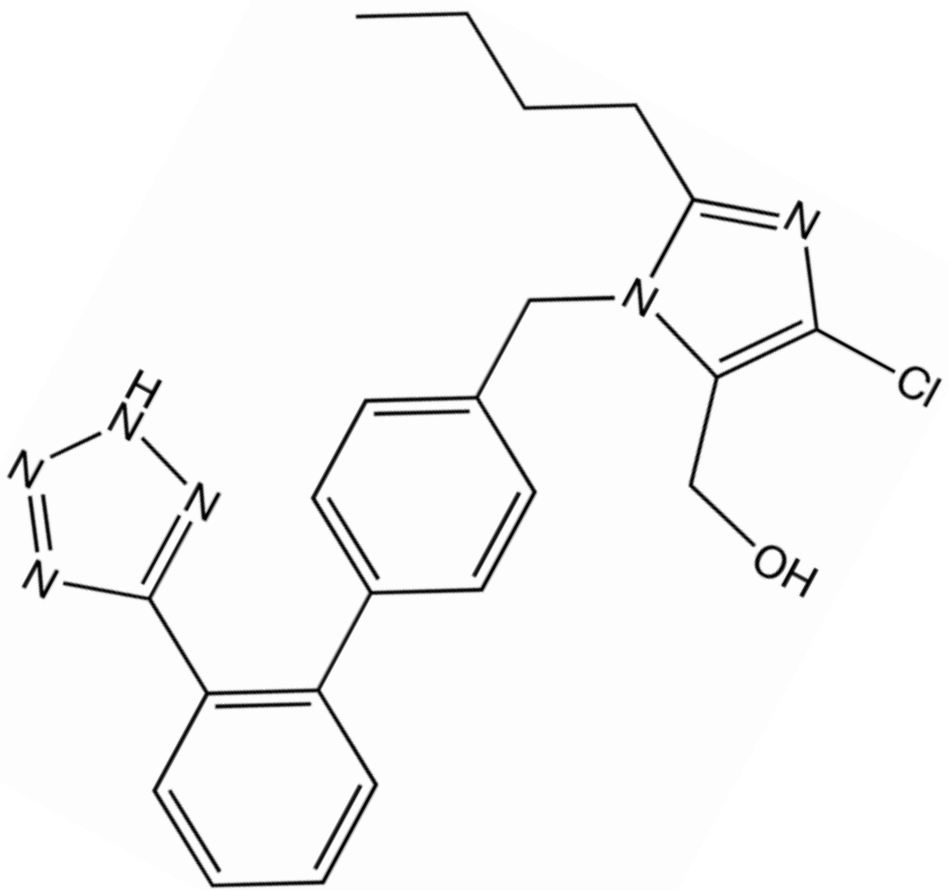
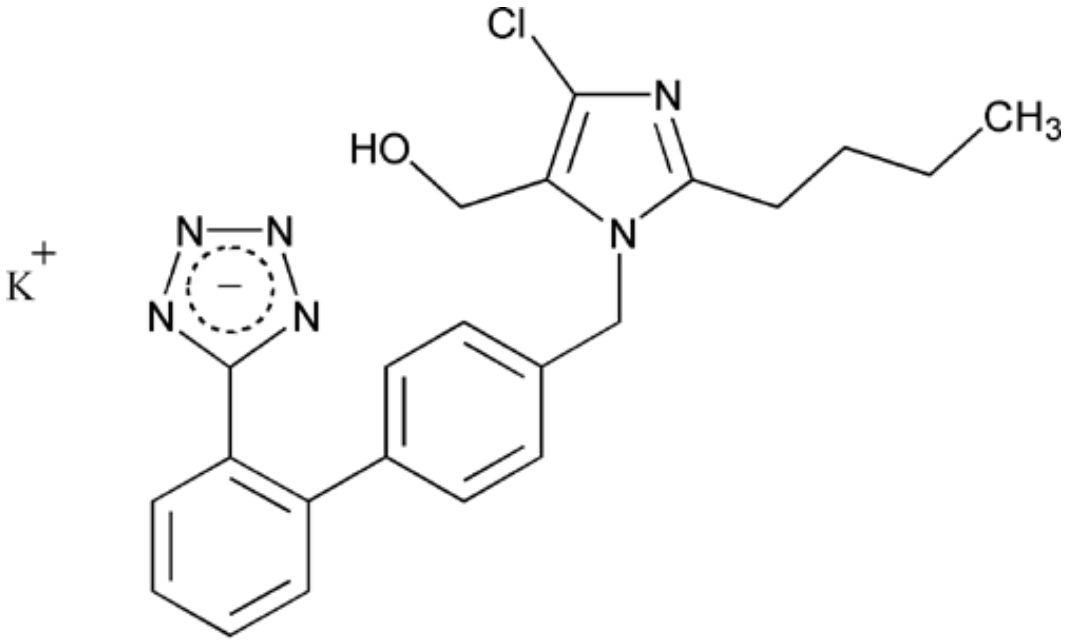
# *Анализ на лекарства – АТ-антагонисти*

# *Losartan*




(1-((2'-(2*H*-tetrazol-5-yl)biphenyl-4-yl)methyl)-
2-butyl-4-chloro-1*H*-imidazol-5-yl)methanol

# *Losartan*




**Identification**

**A:** ***Infrared Absorption*** 197M—

**B:** ***Ultraviolet Absorption*** 197U—

*Solution:* 10 µg per mL.

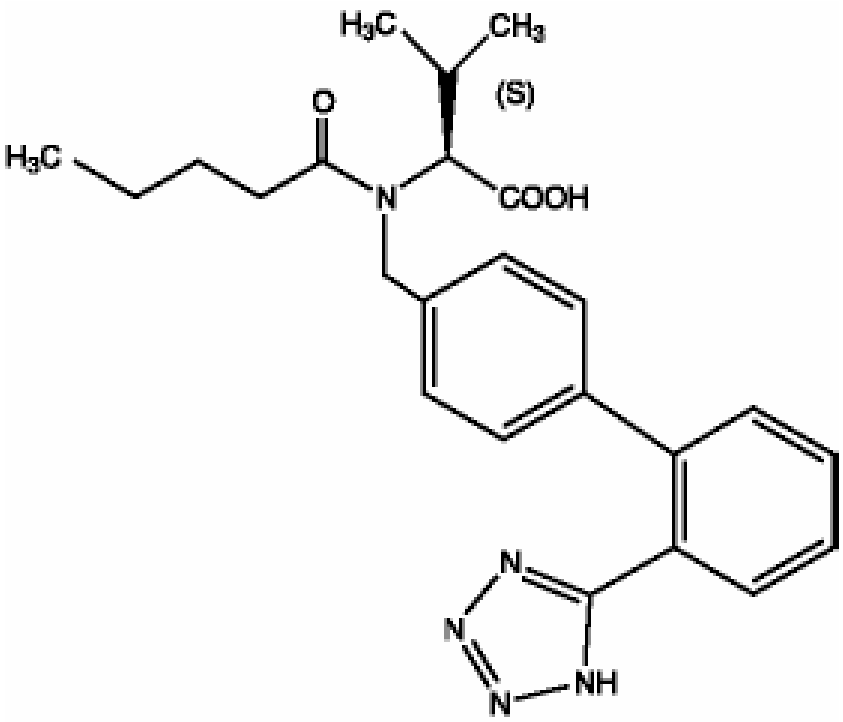
*Medium:* methanol.

**C:** It meets the requirements of the test for *Potassium* 191.

**Water,** *Method I* 921**:** not more than 0.5%.

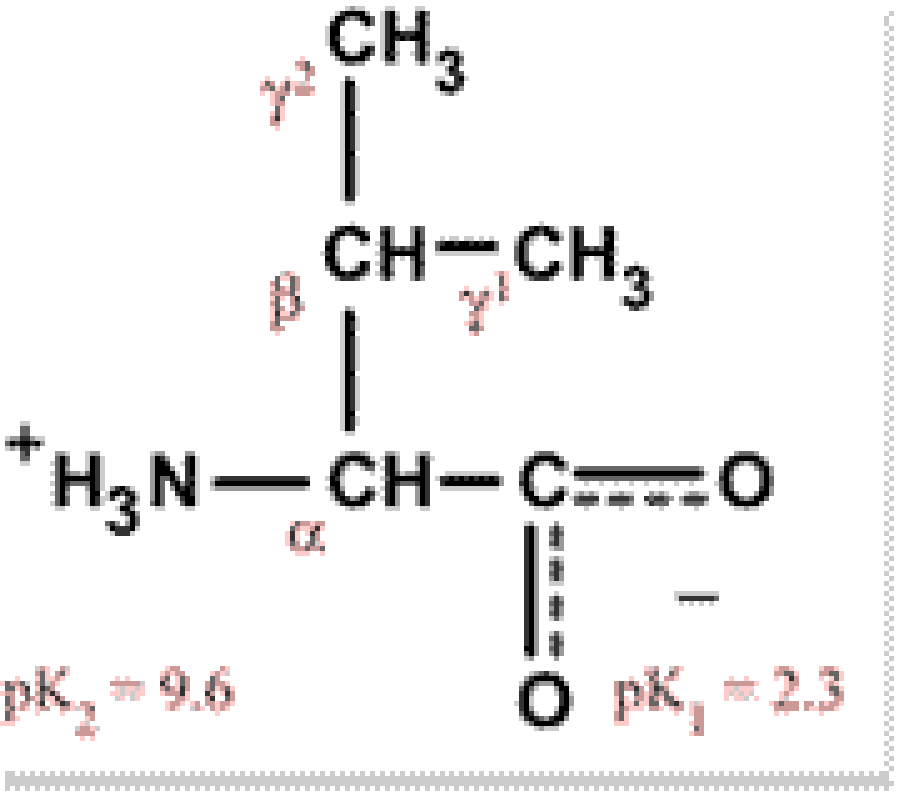
**HPLC Measurements**. Samples were analyzed according to the laboratory standard, HPLC (La-Chrom®, Merck-Hitachi, Darmstadt, Germany) with a diode array detector and a Purospher® STAR RP-18 endcapped column (125-4, 5 µm; Merck, Darmstadt, Germany). The eluent was 45% acetonitril/55% H2O with 0.1% trifluoroacetic acid (pH 1.9) for **losartan and valsartan** and 15% acetonitril/85% $H_2O$ with 0.1% trifluoroacetic acid (pH 1.9) for cefadroxil. UV-detection was done at 232 nm, the injection volume was 20 µl, the flow rate 0.75 ml/min and the column temperature 34°C. Cephalexin was used as internal standard for cefadroxil whereas losartan was used as internal standard for valsartan and vice versa. The retention time were between 2.5 and 8 min.

# Valsartan




*N*-(1-oxopentyl)-*N*-[[2′-(1*H*-tetrazol-5-yl) [1,1′-biphenyl]-4-yl]methyl]-L-valine

# L-valine



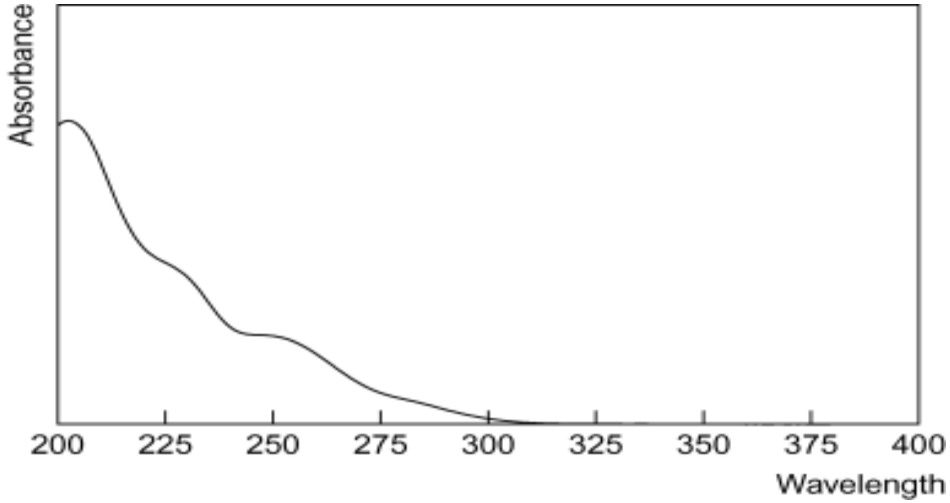
**Gas Chromatography.**
System GC—RI.

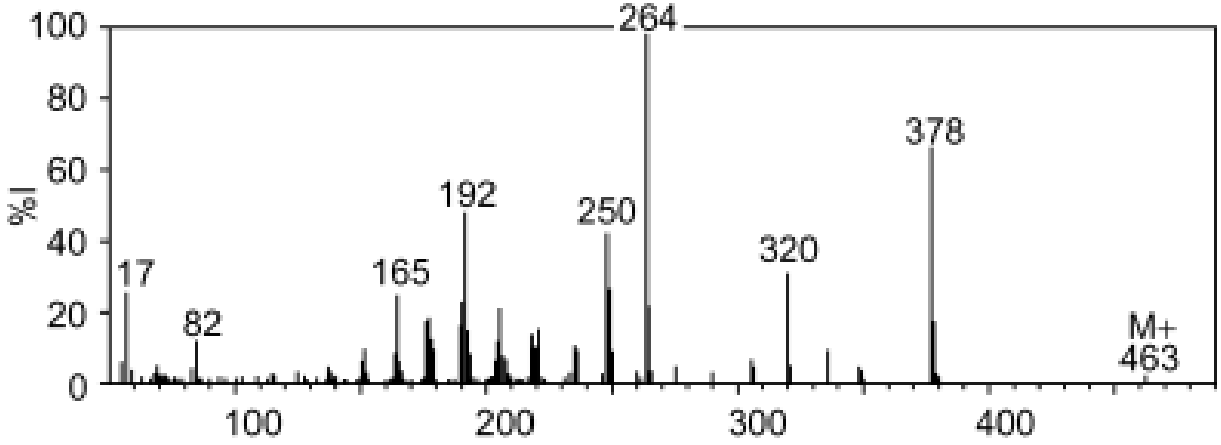
**High Performance Liquid Chromatography.**

Column: **ODS C18** (150 × 4.6 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 2.8):acetonitrile (50:50), flow rate 1.3 mL/min. Fluorescence detection (λex=265 nm; λem=378 nm). Retention time: 5 min.

Column: **Chiral AGP** silica bonded α1-acid glycoprotein (100 × 4 mm i.d., 5 µm). Mobile phase: phosphate buffer (pH 7.0, containing 2% 2–propanol), flow rate 0.8 mL/min. UV detection (λ=227 nm). Retention times: 10 min; CGP 49309, 6 min.

**Ultraviolet Spectrum.**
Aqueous acid—203, 248 nm.



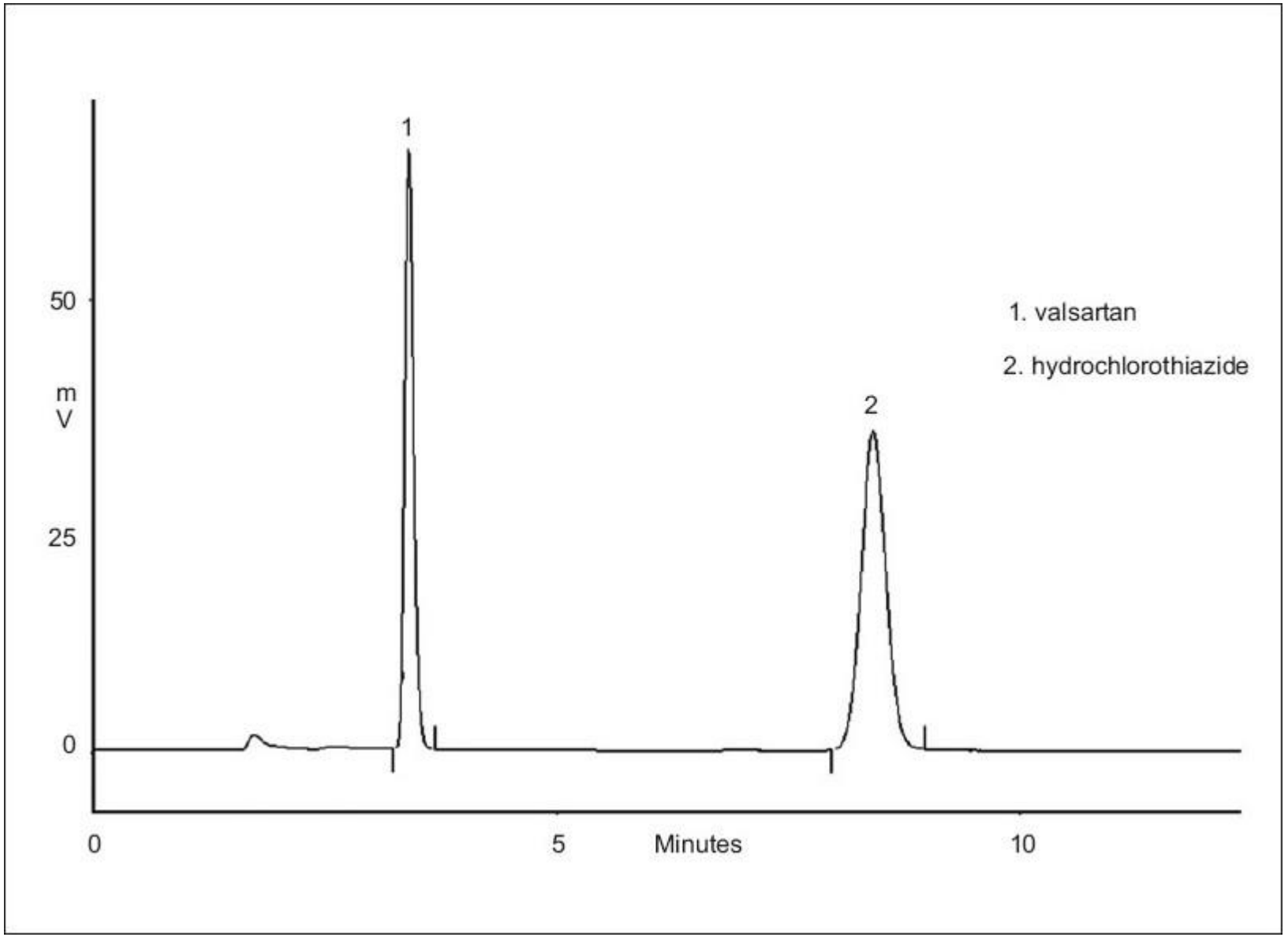




**Mass Spectrum.**
Principal ions at *m/z* 264, 378, 192, 250, 320, 58, 165, 251.

# Simultaneous determination of ***valsartan*** and hydrochlorothiazide in tablets by RP-HPLC

A simple, reproducible and efficient reverse phase high performance liquid chromatographic method was developed for **simultaneous determination of *valsartan* and hydrochlorothiazide in tablets**. A column having 200 × 4.6 mm i.d. in isocratic mode with mobile phase containing methanol : acetonitrile:water:isopropylalcohol (22:18:68:2; adjusted to pH 8.0 using triethylamine; v/v) was used. The flow rate was 1.0 ml/min and effluent was monitored at 270 nm. The retention time (min) and linearity range (µg/ml) for valsartan and hydrochlorothiazide were (3.42, 8.43) and (5-150, 78-234), respectively. The developed method was found to be accurate, precise and selective for simultaneous determination of valsartan and hydrochlorothiazide in tablets.

HPLC Chromatogram of ***valsartan*** and hydrochlorothiazide in tablets



# Isolation and identification of process impurities in crude ***valsartan*** by HPLC, mass spectrometry, and nuclear magnetic resonance spectroscopy

**Abstract**
Three unknown recurring impurities were isolated from crude valsartan by a combination of analytical and preparative liquid chromatography. One of the impurities was identified as (S)-N-valeryl-N-{[2'-(1-methyl-tetrazol-5-yl) biphenyl-4-yl]-methyl}-valine by mass spectrometry and nuclear magnetic resonance spectroscopy. The tentative mechanism for the formation of the impurities is also discussed.

**Journal Title**
Journal of liquid chromatography & related technologies **ISSN** 1082-6076
**CODEN** JLCTFC

**Source**
2006, vol. 29, no1-4, pp. 553-568 [16 page(s) (article)] (17 ref.)

# Biovalidation of an SPE-HPLC-UV-fluorescence method for the determination of ***Valsartan and its metabolite valeryl-4-hydroxy-valsartan*** in human plasma

Gorka Iriarte [1], Nerea Ferreirós [1], Izaskun Ibarrondo [1], Rosa Maria Alonso [*], Miren Itxaso Maguregui [2], Rosa Maria Jiménez

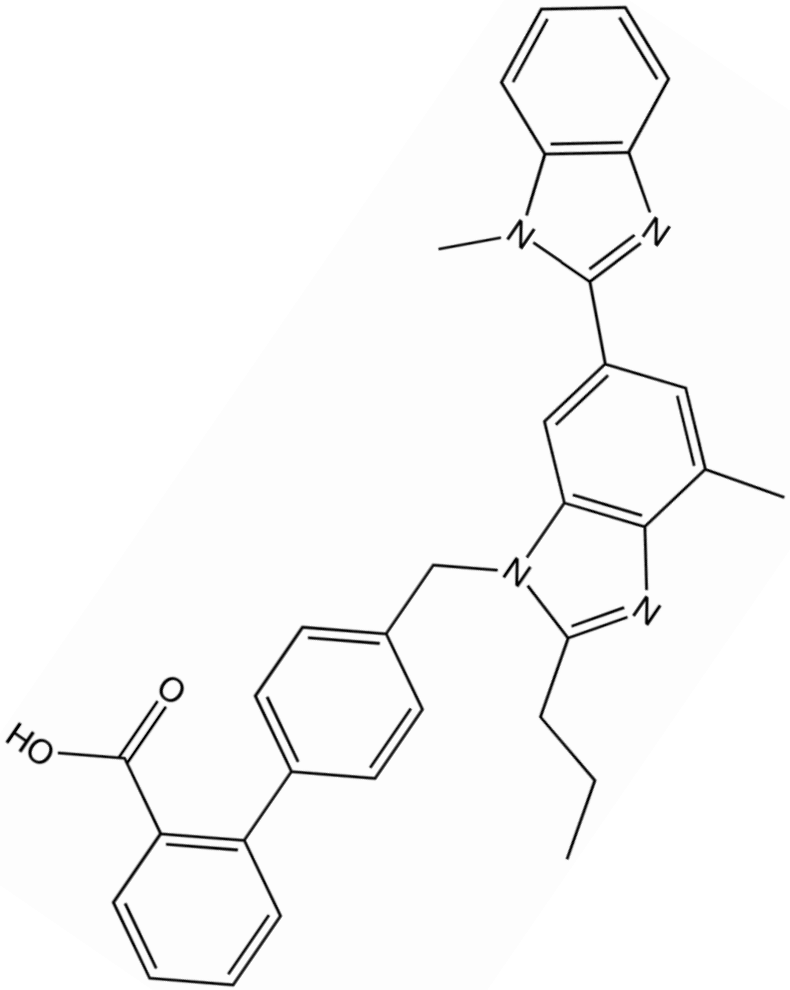
A simple and fast method for the simultaneous determination of the antihypertensive drug ***Valsartan and its metabolite*** in human plasma has been validated. The proposed method deals with SPE, followed by an HPLC separation coupled with fluorimetric and photometric detection. The optimization of the SPE-HPLC method was achieved by an experimental design. The separation was performed on an RP C18 Atlantis 100 mm×3.9 mm column. The mobile phase consisted of a mixture of ACN 0.025% TFA and phosphate buffer (5 mM, pH = 2.5) 0.025% TFA and was delivered in gradient mode at a flow rate of 1.30 mL/min. The eluent was monitored with a fluorescence detector at 234 and 378 nm excitation and emission wavelengths, respectively, and at 254 nm using a photometric detector. The full analytical validation was performed according to the Food and Drug Administration (FDA) guidance for industry: bioanalytical method validation and the recoveries obtained for Valsartan and its metabolite ranged from 94.6 to 108.8%. The validated method was successfully applied to 12 plasma samples obtained from patients under antihypertensive treatment with Valsartan.

Sartans such as **losartan, valsartan, irbesartan and eprosartan** are blockers of the angiotensin II type 1 receptor. They have proven to be effective in the treatment of hypertension, renal diseases, heart failure, ventricular hypertrophy, dilation, arrhythmias anddysfunction with overall reduced cardiovascular morbidity and mortality and less negative side effects than the classical angiotensin converting enzyme inhibitors. Valsartan has gained considerable interest last year when Wang and coworkers (2007) discovered that it lowers brain ß-amyloid protein levels in a mouse model and significantly reduces Alzheimer diseasetype neuropathology and cognitive deterioration, even when delivered at a dose lower than that used for hypertension treatment in humans. After oral administration as the primary route, sartans display bioavailability levels in the range of 13% (eprosartan) to 80% (irbesartan) (Dominiak, 1999). Because some sartans sterically resemble di- and tripeptide derivatives, the question arises whether these drugs interact with peptide transporters.

# Determination of 5- *n* -Butyl-4-{4-[2-( *1H* -tetrazole-5-yl)- *1H* -pyrrol-1-yl] phenylmethyl}-2,4-dihydro-2-(2,6-dichloridephenyl)- *3H* -1,2,4-triazol-3-one, a New Angiotensin Type 1 Receptor Antagonist in Rat Plasma by LC-ESI-MS: Application to Pharmacokinetic Studies

**Abstract** A simple and sensitive reversed-phase LC-ESI-MS method to identify and quantitate **5-*n*-butyl-4-{4-[2-(*1H*-tetrazole-5-yl)-*1H*-pyrrol-1-yl]phenylmethyl}-2,4-dihydro-2-(2,6-dichloridephenyl)-*3H*-1,2,4-triazol-3-one** (1b), a new Angiotensin II type 1 receptor antagonist in rat plasma has been developed and validated. Sample preparation used a simple liquid–liquid extraction with ethyl acetate. Separation was achieved by gradient elution on a C18 column. The mobile phase consisted of acetonitrile and water (0.05% triethylamine and 0.05% acetic acid) at a flow rate of 0.2 mL min−1. The detection utilized selected ion monitoring (SIM) in the negative mode at *m/z* 507.1 and *m/z* 407.2 for the deprotonated molecular ions of 1b and the internal standard irbesartan, respectively. The lower limit of quantification was reproducible at 5 ng mL−1 with 100 μL of plasma and the good linear was observed in the 5–500 ng mL−1 range. This concentration range corresponded well with the plasma concentrations of 1b in pharmacokinetic studies. Recoveries of 1b in rat plasma were 76.1, 74.6 and 79.0% at 5, 50 and 500 ng mL−1. The RSD of intra-assay and inter-assay variations were all less than 5%. This validated LC-ESI-MS assay is an economic, quick, precise and reliable method for the analysis of 1b in pharmacokinetic studies.

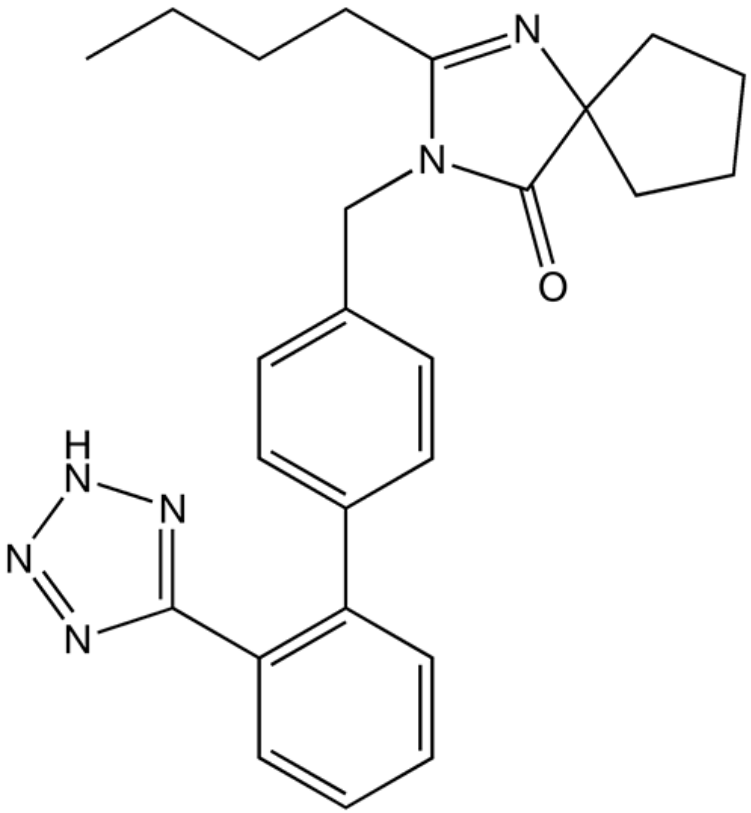
# *Telmisartan*




| Pharmacokinetic data | |
|---|---|
| Bioavailability | 42–100% |
| Protein binding | ≥99.5% |
| Metabolism | Minimal hepatic |
| Half life | 24 hours |
| Excretion | Faecal 97% |

2-[4-[[4-methyl-6-(1-methylbenzoimidazol-2-yl)-2-propyl-benzoimidazol-1-yl]methyl]phenyl] benzoic acid

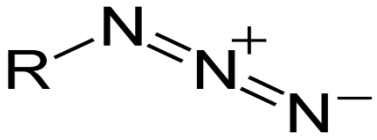
# Irbesartan




2-butyl-3-[*p*-(*o*-1*H*-tetrazol -5-ylphenyl) benzyl]-1,3-diazaspiro[4.4]non-1-en-4-one

**Study on the pharmacokinetics and relative bioavailability of irbesartan capsules in healthy volunteers**

**Summary** The pharmacokinetics and relative bioavailability were studied in 18 healthy volunteers. A single oral dose of 150 mg irbesartan capsule (test) or tablet (reference) was given to each volunteer according to a randomized 2-way crossover study. The concentrations in plasma were determined by **HPLC-UV method**. The main parameters of irbesartan capsules were: Cmax: 1.502±0.295 μg/ml, tmax: 1.44±0.34 h, t1/2: 20.21±14.71 h, AUC0−t; 11.087±3.443 μg/ml−1·h. The relative bioavailability of capsule to tablet was (101.4±28.9)%. The results of statistical analysis showed that two formulations were bioequivalent.



**Limit of azide—**

*Mobile phase*— Prepare a filtered and degassed 0.1 N sodium hydroxide solution (see *System Suitability* under *Chromatography* ⟨621⟩).

*Standard solution*— Transfer about 25 mg of **sodium azide**, accurately weighed, to a 100-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix. Pipet 250 µL of this solution into a 200-mL volumetric flask, dilute with *Mobile phase* to volume, and mix. This solution contains about 0.312 µg of sodium azide per mL.

*Test solution*— Transfer about 100 mg of ***Irbesartan***, accurately weighed, to a 5-mL volumetric flask, dissolve in and dilute with *Mobile phase* to volume, and mix.

*Chromatographic system* (see *Chromatography* ⟨621⟩)— The liquid chromatograph is equipped with a **conductimetric detector**, and a 4.0-mm × 25-cm column that contains packing L46. The flow rate is about 1.0 mL per minute. Chromatograph the

$$2\ NaN_3 + 2\ HNO_2 \rightarrow 3\ N_2 + 2\ NO + 2\ NaOH$$

## Limit of azide

*Standard solution,* and record the peak responses as directed for *Procedure:* the signal to noise ratio for the azide peak is not less than 10.
*Procedure*— Separately inject equal volumes (about 200 µL) of the *Standard solution* and the *Test solution* into the chromatograph, record the chromatograms, and measure the peak area for azide peak. Calculate the amount of azide in ppm in the portion of Irbesartan taken by the formula:

$$1000(CS / CT)(42.02/65.01)(rU / rS),$$

in which *CS* is the concentration, in µg per mL, of sodium azide in the *Standard solution; CT* is the concentration, in mg per mL, of Irbesartan in the *Test solution; rU* is the peak area for azide obtained from the *Test solution;* and *rS* is the peak area for azide obtained from the *Standard solution:* not more than 10 ppm of azide is found.

Azides may be reduced to amines by hydrogenolysis or with a phosphine, e.g. Triphenylphosphin - $Ph_3P$.This reaction allows azides to serve as protected -$NH_2$ synthons: $RN_3 \rightarrow RNH_2$

EMEA/H/C/786

**ЕВРОПЕЙСКИ ДОКЛАД ЗА ОБЩЕСТВЕНА ОЦЕНКА (EPAR)**

**IRBESARTAN BMS**

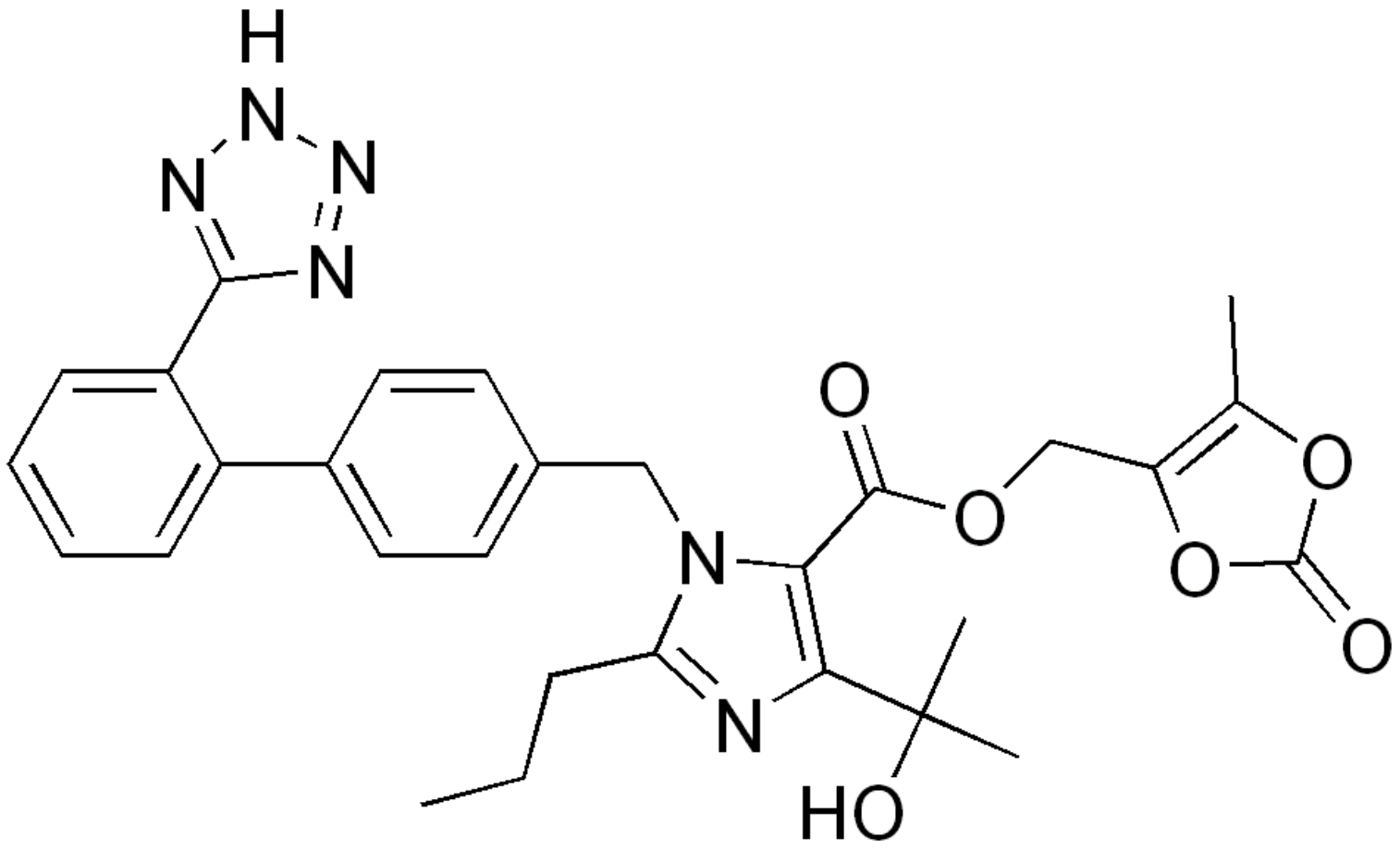
**Резюме на EPAR за обществено ползване**

*Настоящият документ представлява резюме на Европейския доклад за обществена оценка (EPAR). В него се разяснява как Комитетът по лекарствените продукти за хуманна употреба (CHMP) оценява проведените проучвания, за да направи своите препоръки как да се използва лекарството.*

*Ако се нуждаете от повече информация за Вашето медицинско състояние или лечение, прочетете листовката (също част от EPAR) или попитайте Вашия лекар или фармацевт. Ако желаете повече информация за основанията на препоръките на CHMP, прочетете научното обсъждане (също част от EPAR).*

**Какво представлява Irbesartan BMS?**
Irbesartan BMS е лекарство, което съдържа активното вещество ирбесартан. Предлага се под формата на бели овални таблетки (75, 150 и 300 mg).
Това лекарство е същото като Karvea, което вече е одобрено в Европейския съюз (ЕС). Компанията производител на Karvea е дала съгласие научните й данни да се използват за Irbesartan BMS.
**За какво се използва Irbesartan BMS?**
Irbesartan BMS се използва при пациенти с есенциална хипертония (високо кръвно налягане). „Есенциална“ означава, че високото кръвно налягане не е причинено от други заболявания. Irbesartan BMS се използва също за лечение на ренални (бъбречни) заболявания при пациенти с хипертония и диабет тип 2 (неинсулино-зависим диабет). Употребата на Irbesartan BMS не се препоръчва при пациенти на възраст под 18 години.
Лекарствен продукт, отпускан по лекарско предписание.
**Как да използвате Irbesartan BMS?**
Irbesartan BMS се приема през устата със или без храна. Обичайната препоръчвана доза е 150 mg веднъж на ден. Ако не се постигне достатъчен контрол на кръвното налягане, дозата може да бъде увеличена до 300 mg на ден или да се добавят други лекарства за хипертония, например хидрохлоротиазид. При пациенти на хемодиализа (техника за перчистване на кръвта) или при пациенти на възраст над 75 години може да се прилага начална доза от 75 mg.
При пациенти с хипертония и диабет тип 2 Irbesartan BMS се добавя към другите лечения на хипертония. Лечението започва със 150 mg веднъж на ден и дозата обикновено се увеличава до 300 mg веднъж на ден.
**Как действа Irbesartan BMS?**
Активното вещество в Irbesartan BMS, ирбесартан, е „ангиотензин-II рецепторен антагонист“, което означава, че блокира действието на хормона в организма, наречен ангиотензин II. Ангиотензин-II е мощен вазоконстриктор (вещество, което свива кръвоносните съдове). Като блокира рецепторите, с които нормално се свързва ангиотензин II, ирбесартан спира действието на хормона и позволява на кръвоносните съдове да се разширят. Това позволява кръвното налягане да се понижи и намалява рисковете от увреждания, причинени от високото кръвно налягане, например получаване на удар.

## *Olmesartan*




(5-methyl-2-oxo-1,3-dioxol-4-yl)methyl 5-(2-hydroxypropan-2-yl)-2-propyl-3-[[4-[2-(2H-tetrazol-5-yl)phenyl]phenyl]methyl]imidazole-4-carboxylate