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**Research Article**

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# Analytical study of UV-Spectrophotometric and HPLC methods for simultaneously determination of metoprolol and hudrochlorothiazide in fixed-dosage combinations

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**ABSTRACT**

*The aim of this study is developing and validation of two analytical methods (UV-spectrophotometric and HPLC) for determination of Metoprolol tartrate and Hydrochlorothiazide fixed-dosage combinations. The investigation includes validation of procedures for performance of the tests for identification, purity and assay. Analytical parameters precision, accuracy, selectivity, linearity, limit of detection and limit of quantitation were studied and compared. The both methods allow simultaneously determination of Metoprolol tartrate and Hydrochlorothiazide at different conditions (algorithm, wavelength zones, column and mobile phase). The preferences of each of them were estimated on the comparison of analytical parameters from the validation procedures. They let to compose the criteria in accordance with European Pharmacopoeia and EU regulates about the use of official analytical programms for quality control of fixed-dosage drugs. The methods has been applied successfully in analysis of pharmaceutical preparation in the appointed for each of them aspect of applications.*

**Keywords:** Metoprolol tartrate, Hydrochlorothiazide (HCT), antihypertensive drug combination, UV-spectrophotometry, HPLC.

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## INTRODUCTION

Recently antihypertensive drug combinations containing β-blockers and diuretics from thiazide class are used very often in the treatment and the prevention of several diseases of the cardiovascular system. These fixed-dosage combinations generally are not recommended for initial therapy, but they are utilized in maintenance therapy after the required dose is established, in order to increase convenience, economy, and patient compliance. From the other hand their significance and new therapeutical benefits in the clinical therapy increases continuously and acquires so large size that the question of the quality control comes forward.

**Metoprolol** (*RS*)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol) is a selective $\beta_1$ receptor blocker. The active substance metoprolol definite as metoprolol tartrate is an immediate-release formulation. **Hydrochlorothiazide (**6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide), abbreviated **HCT**, is a first-line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water.

The most adequate methods for analysis of Metoprolol tartrate and Hydrochlorothiazide ate UV-spectrophotometric and HPLC methods. In literature there are presented thoroughly methods for their estimation in combinations, pharmaceutical preparations and mixtures [2, 3, 4, 5, 6, 7]. They are adapted to decide rather closed problems [8, 9, 10]. Some of these methods have a validation but it is opened the question about the developing of quality control criteria for identification, purity and assay tests for Metoprolol tartrate and Hydrochlorothiazide in according with European Pharmacopoeia and thus needed for producers of fixed-dosage forms.

The aim of this study is a developing and validation of UV-spectrophotometry and HPLC methods for simultaneously determination of frequently used in many drug formulations Metoprolol tartrate and Hydrochlorothiazide fixed mixtures with high precision, accuracy, selectivity and wide spectra of applications. Analytical and validation criteria are based on investigation of spectrophotometric and chromatographic parameters [1, 11, 12], ICH and European Pharmacopoeia requirements [13, 14].

## EXPERIMENTAL SECTION

### 2.1 UV-spectrophotometric method

**Reagents:** Metoprolol tartrate and Hydrochlorothiazide reference substances (CRS), fixed combination Metoprolol tartrate and Hydrochlorothiazide in concentration ratios 25 : 75, 75 : 25 and 50 : 50 mg respectively.

**System:**
UV/VIS Spectrometer HP;
Diode array detector;
Wavelength Range – 190 – 820 nm;
Wavelenght Accuracy – ± 2 nm;
UV/VIS operating software.

**Analytical Calculations**
Analytical calculations are based on Multicomponent analysis calculations. Method's options are given on table 1.

**Table1. Analytical calculations**

| Calculations | Method's options |
|---|---|
| Analysis | Multicomponent (MCA) |
| Calibration Curve type | Beer's Law |
| Algorithm | Least Squares fit (LSQ) |
| Derivative order | 0 |
| Polynomial Degree | 0 |
| Smoothing Points | 1 |
| Data Interval | 2 nm |
| Analytical Wavelength Zone I | 204 nm to 204 nm |
| Analytical Wavelength Zone II | 190 nm to 300 nm |
| Temperature | 25 $^{o}$C |

**Test preparation:**
- Test solutions from Metoprolol tartrate and Hydrochlorothiazide were prepared by dissolving and mixing of adequate amounts of substances in methanol to obtain solutions with concentrations 2.5, 5.0 and 7.5 mg/ml respectively.
- Reference solutions were prepared by the same manner from CRS.

**Procedure:**
The prepared solutions were tested by normal spectrophotometry in full UV range against blank sample containing methanol. The investigations were carried out at different analytical zones - analytical wavelength zone I from 204 nm to 204 nm and analytical wavelength zone II from 190 nm to 300 nm using operating software.

### 2.2 **HPLC method**

***Chromatographic system*:**
The chromatographic procedure was carried out using:
Liquid chromatograph Shimadzu LC – 10 Advp equipped with 4.6 x 250 mm column Luna 5U C18 (2) 100 A, Phenomenex ODS with particle size 5 µm;
Detector SPD 10 AVvp – UV-VIS with fixed analytical wavelengths.

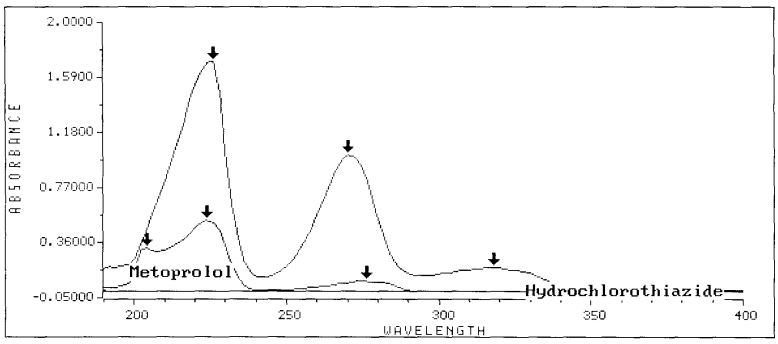
***Chromatographic conditions:***
Isocratic mobile phases, prepared by mixing of filtered and degassed Acetonitril / Phosphate buffer (35 : 65 v/v)
- 226 nm analytical wavelength;
- column temperature 25 $^{0}$C;
- flow rate about 1.5 ml/min.

***Reagents:*** Metanol HPLC grade, Acetonitril HPLC grade, Distilled water R (Reagents (R), European Pharmacopoeia 7.0), Metoprolol tartrate and Hydrochlorothiazide reference substances (CRS), Buffer solution with pH 7.0 prepared by European Pharmacopoeia, 7.0. All reagents are analytical grade quality.

**Test preparations:**
*Solution of metoprolol tartrate (a)*: 0.0100 g Metoprolol tartrate were dissolved in 100.0 ml flask with a solvent mixture (mobile phase). The concentration of obtained solution is 0.0001 g/ml;
*Solution of Hydrochlorothiazide (b)*: 0.0100 g Hydrochlorothiazide reference substance were dissolved in 100.0 ml flask with a solvent mixture (mobile phase). The concentration of obtained solution is 0.0001 g/ml;
*Reference solutions* were prepared by the same manner using Metoprolol tartrate CRS - *solution (c)* and Hydrochlorothiazide CRS – *solution (d)*;
*Test solution (e)* contains aliquot parts (volume/volume) from solutions (a) and (b) dilute so to obtain solutions with concentration ratios 2.5 : 7.5, 7.5 : 2.5 and 5.0 : 5.0 mg respectively.

## RESULTS AND DISCUSSION

***Validation of UV-spectrophotometric method:***
Selectivity:
Using MCA selectivity was achieved measuring by normal spectrophotometry in two analytical wavelength zones – at 204 nm and from 190 to 300 nm. On Fig. 1. is shown UV-spectra of Metoprolol tartrate and Hydrochlorothiazide mixture solution in methanol with concentration 5 mg/ml for each of both components.
Fig. 1. UV-spectra of Metoprolol tartrate and Hydrochlorothiazide model mixture.




*Precision:*
Seven (7) equal solutions from homogenous samples containing Metoprolol tartrate and Hydrochlorothiazide in concentration ratio 1 : 1 were analyzed by UV-spectrophotometric method. Standard deviation (SD) and relative SD (RSD) were found. The results are presented on Table 2.

**Table 2. Precision of samples containing. Metoprolol tartrate and Hydrochlorothiazide (HCT) in concentration ratio 50 : 50 (mg)**

| N | Amount of Metoprolol tartrate (mg) | Amount of HCT (mg) | $X_{mean}$ (mg) | SD (mg) | SD (%) |
|---|---|---|---|---|---|
| 1 | 53,7930 | 50,7280 | For Metoprolol tartrate: 54.5195 | For Metoprolol tartrate: 3.095 | For Metoprolol tartrate: 5.67 |
| 2 | 58,7200 | 46,6300 | | | |
| 3 | 51,0450 | 49,2070 | | | |
| 4 | 51,8130 | 51,9030 | For HCT 49.1381 | For HCT 1.876 | For HCT 3.81 |
| 5 | 54,4560 | 47,0090 | | | |
| 6 | 58,7400 | 49,4700 | | | |
| 7 | 53,0700 | 49,0200 | | | |

*Accuracy*
Model mixtures of solutions containing Metoprolol tartrate and Hydrochlorothiazide in concentration ratio 50 – 150 % of theoretical calculated quantity were prepared and analyzed three times each. The results are shown on Tables 3. They are presented as % recovery and SD (mg). At fixed analytical parameters only combination with ratio 50 : 50 mg responds to ICH and Pharmacopoeia requirements about accuracy tolerance. Independently of low values of SD the recovery of Metoprolol tartrate in concentration ratios 25 : 75 and 75 : 25 mg exceeds the tolerance limits.

**Table 3. Accuracy of samples containing Metoprolol tartrate and Hydrochlorothiazide in concentration ratios 25 : 75, 75 : 25 and 50 : 50 mg respectively.**

| Putted amounts (1)* : (2)* (mg) | $X_{mean}$ from found amounts (mg) | SD (mg) | Recovery (%) |
|---|---|---|---|
| 25 : 75 | (1)* 36,0610<br>(2)* 75,8990 | +/- 1,1418<br>+/- 0,4168 | (1)* 144.24<br>(2)* 101.19 |
| 75 : 25 | (1) 94,3670<br>(2) 27,2470 | +/- 3,0122<br>+/- 1,0986 | (1) 125.82<br>(2) 108.98 |
| 50 : 50 | (1) 51,0735<br>(2) 49,0055 | +/- 1,8675<br>+/- 0,6722 | (1) 102.14<br>(2) 98.01 |

*(1)* Metoprplol tartrate, (2)* Hydrochlorothiazide (HCT)*

*Limit of detection:*
1 µg for Metoprplol tartrate and 2.5 µg for Hydrochlorothiazide, established on the base of ratio noise – signal – 1:3.

*Limit of quantitation:*
25 µg for Metoprplol tartrate and 25 µg for Hydrochlorothiazide, established on the base of ratio noise – signal – 1:10.

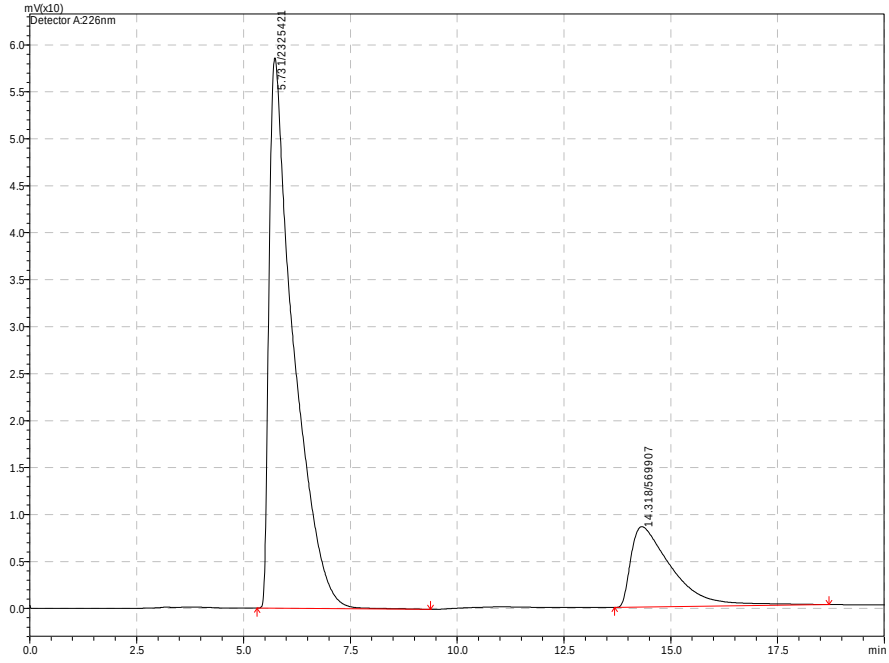
*Linearity:*
The analytical parameter linearity was studied in concentration ratio 1 µg – 10 mg. The accordance between the Absorption, measured in absorption units (AU) and concentrations in g/ml is proportional in the intervals. The correlation coefficients were found to be about 1.

**Table 4. Results from analytical parameters obtained by UV-spectrophotometric and HPLC methods.**

| **Parameter** | **UV-spectro-photometric method** | **HPLC method** |
|---|---|---|
| **Selectivity** | positive | positive with resolution – 2.86 |
| **Precision (RSD %)** | (1)* - 5.67 %<br>(2)* - 3.81 % | (1) - 2.00 %<br>(2) - 1.24 % |
| **Accuracy (Recovery %)** | (1) - 102.14 %<br>(2) - 98.01 % | (1) - 99.33 %<br>(2) - 100.93 % |
| **LOD (µg)** | (1) - 1µg<br>(2) - 2.5 µg | (1) - 0.1µg<br>(2) - 0.25 µg |
| **LOQ (µg)** | (1) - 25 µg<br>(2) - 25 µg | (1) - 10 µg<br>(2) - 10 µg |
| **Linearity interval** | 1 µg – 10 mg | commensurable |

*(1)* Metoprplol tartrate, (2)* Hydrochlorothiazide (HCT)*

**Fig. 2. Chromatogram of Metoprolol tartrate and Hydrochlorothiazide model mixture in concentration ratio 7.5 : 2.5 mg.**



***HPLC determination of Metoprolol tartrate and Hydrochlorothiazide model mixtures:***
For the development of the validation procedure of HPLC methods the following analytical and chromatographic parameters were studied: selectivity, repeatability, accuracy, limit of detection, limit of quantitation, linearity and resolution. The obtained data from the procedure were compared with those obtained from UV-spectrophotometric method. The results are presented in Table 4. On fig. 2 is shown chromatogram of Metoprolol tartrate and Hydrochlorothiazide model mixture in concentration ratio 7.5 : 2.5 mg at prescribed chromatographic conditions. The results respond to ICH and Pharmacopoeia requirements about validation parameters limits.

The tested procedures by above UV-spectrometric and HPLC methods for Metoprolol tartrate and Hydrochlorothiazide - substance mixtures and in drug preparations with fixed doses are for identification, purity and assay tests. For identification and purity all of studied methods are suitable but doubtless HPLC method show higher selectively and performance and answer the requirements about system suitability test (better selectivity, LOD and LOQ values). LOD values are comparatively of the order of µg. Assays test depends prior to precision, accuracy, LOQ and linearity. The results obtained from different methods are similar and then no reason to use the buffered mobile phase except of negative selectivity and availability of impurities. In the cases when analysts will be obtained relative comparatively results the estimation is based on the studies for selectivity, linearity and resolution. These studies lead not only to an exact assessment but they make analysis more ensure and adequate to real state.

## CONCLUSION

The conditions of novel UV-spectrophotometry method for simultaneously determination of Metoprolol tartrate and Hydrochlorothiazide in drug preparation with different dose ratios were performed. The method was studied and validated in complains with European Pharmacopoeia criteria and it distinctive properties are high selectivity, optimal values of analytical parameters, simplicity and wide spectra of applications for purposes of pharmaceutical practices. HPLC procedure for quality control of Metoprolol tartrate and Hydrochlorothiazide was performed and method is validated in respect of purposes of pharmaceutical practice for binary mixtures purity and identification tests.

The methods are filled each other in respect of complete analytical procedure for quality control of Metoprolol tartrate and Hydrochlorothiazide fixed-dosage forms.

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