Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2013, 5(7):132-140

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Chemical stability-indicating HPLC study of fixed-dosage combination containing metoprolol tartrate and hydrochlorothiazide

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ABSTRACT

Chemical stability-indicating HPLC study of fixed-dose combination containing Metoprolol tartrate and Hydrochlorothiazide was developed and kinetic behavior of drugs was appointed and presented in respect of different pH (acid media with pH = 2.0, neutral media with pH = 7.4 and alkaline media with pH = 9.0) and time. The HPLC method was validated according to European Pharmacopoeia requirements and chromatographic and analytical parameters specificity, reproducibility, accuracy, LOD, LOQ and system suitability test were studied.

Key words: Metoprolol tartrate, Hydrochlorothiazide, fixed-dose combination, HPLC, chemical stability

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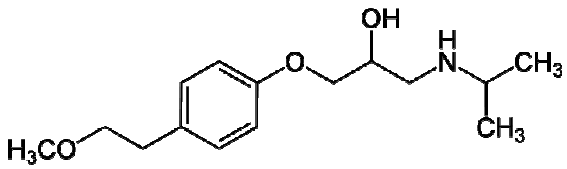
INTRODUCTION

Metoprolol ((*RS*)-1-(isopropylamino)-3-[4-(2-methoxyethyl) phenoxy] propan-2-ol) (fig. 1) is a selective β_1 receptor blocker used in the treatment of several diseases of the cardiovascular system, especially hypertension. The active substance metoprolol is employed either as metoprolol succinate or metoprolol tartrate (where 100 mg metoprolol tartrate corresponds to 95 mg metoprolol succinate). The tartrate is an immediate-release and the succinate is an extended-release formulation [1]. Metoprolol undergoes a-hydroxylation and O-demethylation as a substrate of the cytochrome liver enzymes CYP2D6 and a small percentage by CYP3A4 [2].

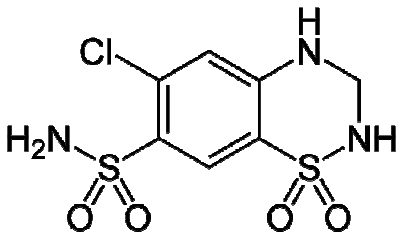
Fig. 1. Chemical structure of metoprolol.



Hydrochlorothiazide (6-chloro-1,1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide), abbreviated **HCTZ**, **HCT**, or **HZT**, is a first-line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart and thus cardiac output and, by other mechanisms, is believed to lower peripheral vascular resistance [3]. Hydrochlorothiazide is a calcium-sparing diuretic, meaning it can help the body get rid of excess water while still keeping calcium.

Hydrochlorothiazide is frequently used for the treatment of hypertension, congestive heart failure, symptomatic edema, diabetes insipidus, renal tubular acidosis, and the prevention of kidney stones [4]. Hydrochlorothiazide belongs to the thiazide class of diuretics. It reduces blood volume by acting on the kidneys to reduce sodium (Na) reabsorption in the distal convoluted tubule. The major site of action in the nephron appears on an electroneutral Na+-Cl- co-transporter by competing for the chloride site on the transporter. By impairing Na transport in the distal convoluted tubule, hydrochlorothiazide induces a natriuresis and concomitant water loss. Thiazides increase the reabsorption of calcium in this segment in a manner unrelated to sodium transport [5]. Additionally, by other mechanisms, HCTZ is believed to lower peripheral vascular resistance [6].

Fig. 2. Chemical structure of hydrochlorothiazide.



Recently the both active substances present in different fixed-dosage drug combinations. Metoprolol tartrate and hydrochlorothiazide combinations are indicated for the management of hypertension but they are not indicated for the initial therapy.

When the fixed combination represents the dose titrated to the individual patient's needs, therapy may be more convenient than with the separate components. Because combination products involve components that would normally be regulated under different types of regulatory authorities, they raise challenging regulatory, policy, and review management challenges. Differences in regulatory pathways for each component can impact the regulatory processes for all aspects of product development and management, including preclinical testing, clinical investigation, marketing applications, manufacturing and quality control, adverse event reporting, promotion and advertising, and post-approval modifications [7-12]. The combination Metoprolol tartrate Hydrochlorothiazide have been presented in U.S. Pharmacopoeia as a tablet form. Applied methods for identification, purity tests and assay define active ingredients separately by different ways [13-15]. In the scientific literature there are reported several HPLC and UV-spectrophotometric methods applied for assay, isomer separation, bioavailability and determination of each of both drugs in drug formulations and human plasma alone or in combination [16,17]. The methods are validated but they solve particular problems with difficult application on quality control process. By means of profound analysis about chemical stability and pharmacokinetic properties is possible to complete fixed-dosage combination drug profile of Metoprolol tartrate / Hydrochlorothiazide and respond to requirements of European Pharmacopoeia and ICH.

Aim: The aim of this study is a development of chemical stability profile of Metoprolol and Hydrochlorothiazide in fixed-dosage combination at acid, neutral and alkaline media using HPLC method.

EXPERIMENTAL SECTION

Material and Methods: HPLC methods; Metoprolo and Hydrochlorothiazide – reference substances, European pharmacopoeia buffer solutions with pH = 2, 7,4 and 9.

***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 wave lengths.

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

Reagents: Metanol HPLC grade, Distilled water R (Reagents (R), European Pharmacopoeia 7.0), Deionized water R (Reagents (R), European Pharmacopoeia 7.0), reference substances Metoprolol tartrate CRS, and Hydrochlorothiazide CRS, Phosphate Buffer with pH = 2, 7,4 and 9 pH units.

Drugs: Metoprolol tartrate, Hydrochlorothiazide, fixed-dose combination containing 100 mg Metoprolol tartrate and 25 mg Hydrochlorothiazide.

Preparation of reference solutions:
- Reference solution (a) of metoprolol tartrate was prepared by dissolving of equivalent amount of Metoprolol tartrate CRS in the mobile phase to obtain solution with concentration 0.00004 g/ml.
- Reference solution (b) of hydrochlorothiazide was prepared by dissolving of equivalent amount of hydrochlorothiazide CRS in the mobile phase to obtain solution with concentration 0.00004 g/ml.
Model mixtures:
- Model mixture I containing 0.01 g metoprolol tartrate RS and 0.0025 g hydrochlorothiazide RS in 50.0 ml solvent mixture (mobile phase).
- Model mixture II containing 0.02 g metoprolol tartrate RS and 0.005 g hydrochlorothiazide RS in 50.0 ml solvent mixture (mobile phase).
- Model mixture III containing 0.03 g metoprolol tartrate RS and 0.0075 g hydrochlorothiazide RS in 50.0 ml solvent mixture (mobile phase).

Test preparation:
To a accurately weighted sample from the fixed-dose combination containing 0.02 g metoprolol tartrate and 0.005 g hydrochlorothiazide was added 50.0 ml buffer solution with pH = 2, 7.4 or 9 pH units. The obtained test solutions were heated at fixed temperature 37^0 C and continuously stirring. In a time 0.1 ml of the samples is taken, diluted to 10.0 ml with the mobile phase and injected into the chromatograph. The study was prolonged to the obtaining of unchangeable remainder.

RESULTS AND DISCUSSION

Validation of HPLC method for studying chemical stability of Metoprolol tartrate and Hydrochlorothiazide in fixed-dosage drug combination:

1. Specificity
Specificity in respect of reagents – “Placebo” solution containing all reagents without active substances was prepared. There no peaks in the chromatogram obtained from this solution with Rt of metoprolol tartrate and Rt of hydrochlorothiazide.

2. Repeatability.
Six (6) equal solutions from homogenous samples containing metoprolol tartrate and hydrochlorothiazide were analyzed by HPLC method. Standard deviation (SD) and relative SD (RSD) were found based on using Area values in absorption units (AU). The RSD for metoprolol tartrate is +/- 2.0 % and for hydrochlorothiazide – 1.34 %.

3. Accuracy
Three model mixtures I, II and III of solutions containing metoprolol tartrate and hydrochlorothiazide in concentration ratio 50 – 150 % of theoretical calculated quantity were prepared and analyzed three times each. The obtained results are shown on table 2.

Table 2. Accuracy of mixtures containing metoprolol tartrate and hydrochlorothiazide.

Model mixtures	Putted amounts (g)	Obtained areas (AU) metoprolol tartrate	hydrochlorothiazide	RSD (%)	Recovery (%)
I	0.01 g metoprolol tartrate / 0.0025 g hydrochloro-thiazide	2102350 2145253 2116470	17106 17350 18325	3.66	99.33 – 100.93
II	0.02 g metoprolol tartrate / 0.005 g hydrochloro- thiazide	3302482 3443409 3478195	245200 234575 246566	2.71	
III	0.03 g metoprolol tartrate / 0.0075 g hydrochloro-thiazide	4464696 4389690 4419090	543807 557446 542158	1.53	

4. Limit of detection:
0.1 μg for metoprolol tartrate and 0.25 μg for hydrochlorothiazide, established on the base of ratio noise – signal – 1:3.

5. Limit of quantitation:
10 μg for metoprolol tartrate and 10 μg for hydrochlorothiazide, established on the base of ratio noise – signal – 1:10.

6. Linearity:
The analytical parameter linearity was studied in concentration ratio 1 μg – 10 mg. The accordance between the Area of peaks, measured in absorption units (AU) and concentrations in g/ml is proportional in the intervals. The correlation coefficients were found to be about 0.99 for metoprolol tartrate and for hydrochlorothiazide.

7. System suitability test.
For system suitability test determination some chromatographic parameters such as *retention time* in different solvents (mobile phase and buffer solutions), *resolution*, *column efficiency* as *number of theoretical plates*, *LOD* and *LOQ* were appointed for optimization of conditions. The results are shown on table 3.

Table 3. Results from system suitability test for fixed-dose combination containing 0.02 g metoprolol tartrate and 0.005 g hydrochlorothiazide in mobile phase solution and buffer media with pH 2.0, 7.4 and 9.0 pH units.

Parameter	Metoprolol tartrate	Hydrochloro-thiazide
Rt (retention time) +/- SD in mobile phase solution	5.76 +/- 0.37	14.94 +/- 0.57
Rt (retention time) +/- SD in buffer solution with pH 2.0	5.61 +/- 0.08	10.95 +/- 0.48
Rt (retention time) +/- SD in buffer solution with pH 7.4	6.01 +/- 0.17	15.79 +/- 1.28
Rt (retention time) +/- SD in buffer solution with pH 9.0	5.86 +/- 0.20	15.11 +/- 0.86
Rs (resolution) +/- SD	1.83+/- 0.14	
N (theoretical plates) (N mean)	274-630	
Tailing factors	0.8	1.27
LOD (limit of detection)	0.10 μg	0.25 μg
LOQ (limit of quantitation)	10 μg	10 μg

HPLC in vitro chemical stability:
The chemical stability of Metoprolol and Hydrochlorothiazide was studied by validated HPLC method at varied conditions: pH and time. The results are shown on Table 4 and Table 5. Stability data was based on chromatographic parameters Rt, Area and Height values at different pH in time intervals 300 min for the investigation in acid media, 240 min in neutral media and 180 min in alkaline media.

HPLC analysis for chemical stability of metoprolol tartrate and hydrochlorothiazide show as follow:

- at pH 2.0 – in the strong acid media the concentration of metoprolol tartrate is exponential function of the time. It is increased gradually and the reaction we observed is the first-order reaction (Fig 3). The Rt of metoprolol tartrate is varied in interval 5.49 – 5.70 min which is in SD values. The regression is linear and the correlation coefficient R is

0.98502 for metoprolol tartrate kinetic reaction. The total sum of related substances is not exceeded 1.42 % from total quantity (Fig. 6).

Table 4. Stability data of Metoprolol tartrate at different pH and time.

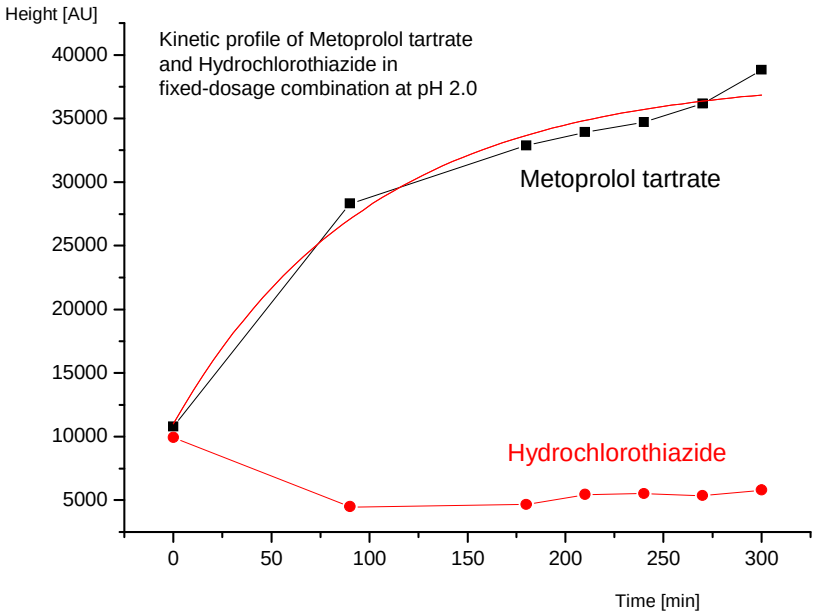
Time (min)	Rt (min)	Area	Height	pH
0	5.492	481396	10788	2
30	5.514	1462828	36081	2
60	5.524	1272179	32131	2
90	5.531	1123844	28294	2
120	5.659	1532562	38571	2
180	5.654	1224924	32874	2
210	5.671	1426957	33920	2
240	5.697	1433117	34696	2
270	5.696	1378120	36166	2
300	5.700	1460661	38807	2
0	5.910	704160	17956	7.4
30	5.843	1118992	28270	7.4
60	5.801	1253056	31733	7.4
90	5.807	1202308	30395	7.4
120	5.922	1213597	30633	7.4
150	6.029	1484214	31178	7.4
180	6.091	1793181	32125	7.4
210	6.228	1304251	32742	7.4
240	6.275	1239752	31009	7.4
0	5.747	1857015	46587	9
60	5.683	2658767	60137	9
90	5.731	2325421	58597	9
120	5.810	2357441	59076	9
150	6.105	2860911	64568	9
180	6.138	2632557	65948	9

Table 5. Stability data of Hydrochlorothiazide at different pH and time.

Time (min)	Rt (min)	Area	Height	pH
0	10.419	636626	9916	2
30	10.503	450688	7022	2
60	10.541	346219	5237	2
90	10.513	274136	4464	2
120	10.485	362172	5690	2
180	11.500	294662	4662	2
210	11.449	341914	5443	2
240	11.429	342432	5505	2
270	11.377	331802	5356	2
300	11.306	356030	5797	2
60	15.761	266860	3727	7.4
90	15.700	254050	3602	7.4
120	14.832	255138	3879	7.4
150	14.420	311859	4741	7.4
180	14.779	374877	5250	7.4
210	17.663	259491	3675	7.4
240	17.398	250091	3509	7.4
0	14.351	575160	8353	9
60	14.683	629474	9488	9
90	14.318	569907	8554	9
120	14.989	543141	7771	9
150	16.288	670248	9736	9
180	16.078	567598	8911	9

Hydrochlorothiazide is relatively stable in the buffer solution with pH = 2.0 pH units in all investigated time interval of 300 min. The concentration of compound is varied from about 100 % to 90.93 % from theoretically estimated.

Fig. 3. Kinetic profile of fixed-dosage combination containing metoprolol tartrate and hydrochlorothiazide at pH 2.0.



- at pH 7.4 and pH 9.0 – In the phosphate buffer media with pH = 7.4 and borate buffer media with pH = 9 metoprolol tartrate and hydrochlorothiazide are stable for 240 and 180 minutes respectively (Fig. 4 and 5). The used conditions allowed simultaneous determination of substances. The relative retention between metoprolol tartrate and hydrochlorothiazide is 2.67 for neutral media and 2.51 for alkaline media and permits propriety in the purity tests. On the chromatograms related peaks of metoprolol tartrate and hydrochlorothiazide aren't observed (Fig. 7 and 8).

Fig. 4. Kinetic profile of fixed-dosage combination containing metoprolol tartrate and hydrochlorothiazide at pH 7.4.

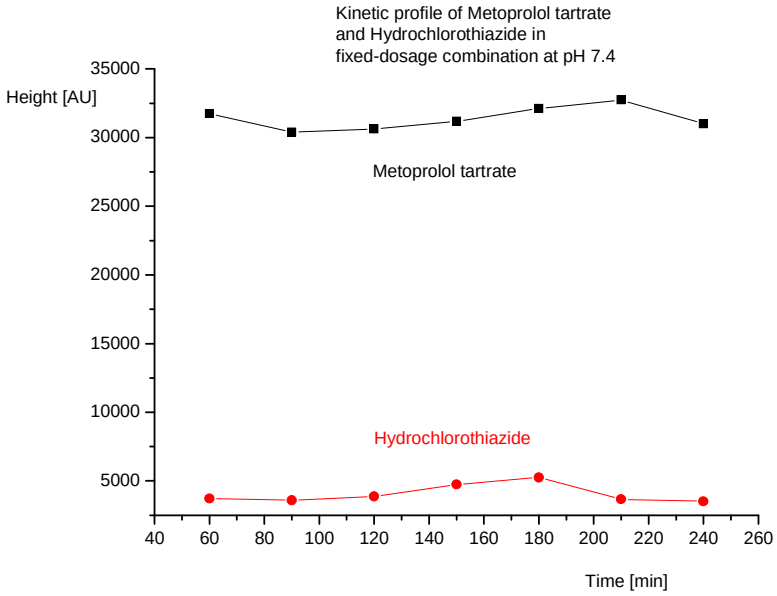


Fig. 5. Kinetic profile of fixed-dosage combination containing metoprolol tartrate and hydrochlorothiazide at pH 9.0.

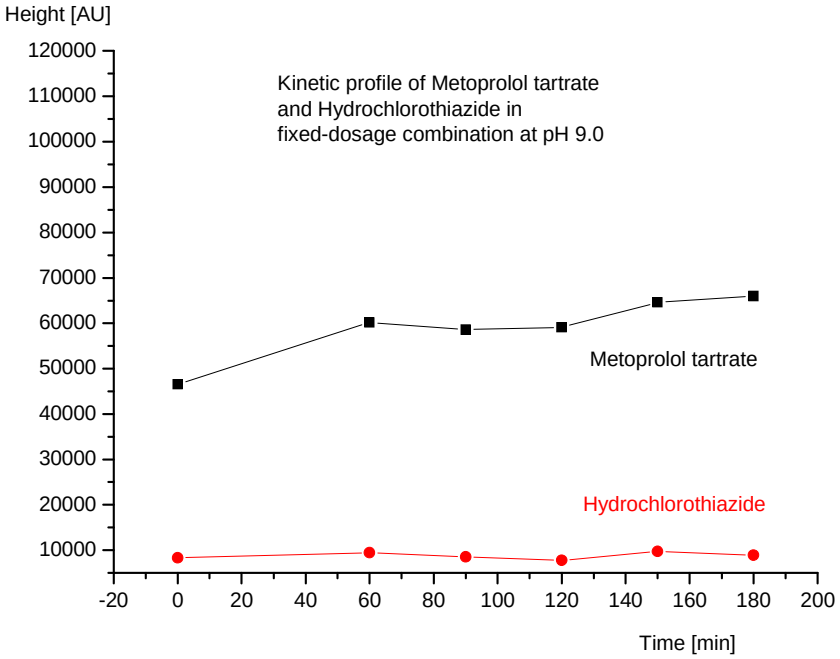


Fig. 6. Chromatogram of metoprolol tartrate and hydrochlorothiazide in fixed-dosage combination at pH = 2.0.

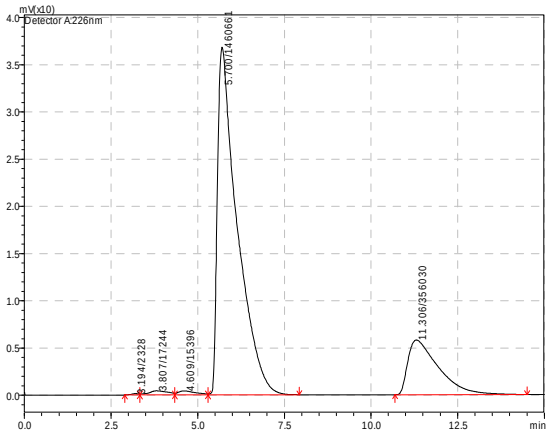


Fig. 7. Chromatogram of metoprolol tartrate and hydrochlorothiazide in fixed-dosage combination at pH = 7.4.

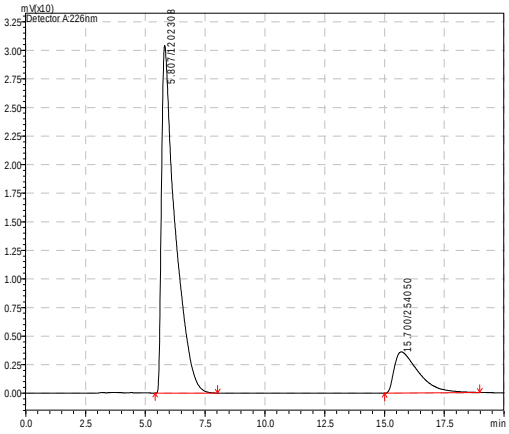
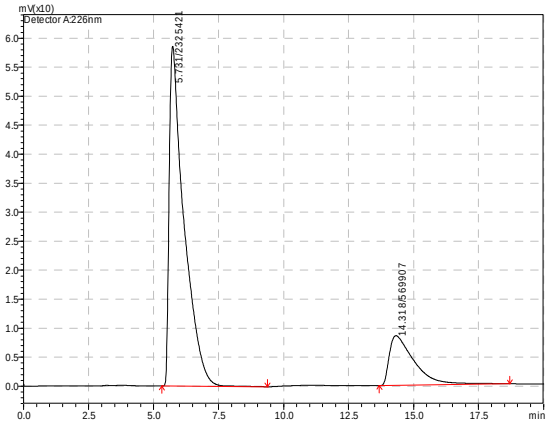


Fig. 8. Chromatogram of metoprolol tartrate and hydrochlorothiazide in fixed- dosage combination at pH = 9.0.



CONCLUSION

The chemical stability profile of Metoprolol tartrate and Hydrochlorothiazide was developed in acid, neutral and alkaline media with validated HPLC method. The kinetic behavior of drugs was appointed and presented as analytical model for monitoring. The obtained data can serve in creating of in vivo monitoring program and quality control of different drug preparations.

Acknowledgement
This study is a part of Grant project and is supported by Medical University – Sofia, Medicine Science Council.

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