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Original Article

Preparation of Combination Tablet Containing Lisinopril (10mg)/ Hydrochlorothiazide (12.5mg): Characterization and Stability studies

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Hanieh Mousavie[1], Mohammad Reza Avadi[2,3*], Assal M.M.Sadeghi[3], Arash Mahboubi[4], Orkideh Ghorban Dadras[5]

[1] *Department of Pharmaceutics, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran*

[2] *Department of Nanotechnology, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran*

[3] *Hakim Pharmaceutical Company, Tehran, Iran*

[4] *Department of Pharmaceutics, Shaheed Beheshti University of Medical Sciences, Tehran, Iran*

[5] *Department of Medicinal Chemistry, Pharmaceutical Sciences Branch, Islamic Azad University (IAUPS), Tehran, Iran*

Abstract

In modern clinical practice, combination fixed - dose tablets have become a baseline for effective therapy in many clinical conditions. The Hydrochlorothiazide (Hctz) (12.5mg) / Lisinopril (Lis) (10 mg) tablet is a combination drug for treatment of hypertension. In this study, the tablets were prepared by wet granulation method and characterized in terms of weight variation, hardness, friability, disintegration time, dissolution and stability. A high-performance liquid-chromatographic method (HPLC) was used to assay Hctz and Lis in the prepared tablets. Amongst different formulations, WG14 was found to be the best for product development based on both physicochemical and stability results. Pharmaceutical characteristics of the tablets were compared with the Innovator tablet 1A-Pharma (Germany).

Keywords: Combination Tablet, Lisinopril, Hydrochlorothiazide, Characterization, Stability

***Corresponding author**: Mohamad Reza Avadi, Pharm D.,PhD. Department of Pharmaceutics, School of Pharmacy, Islamic Azad University-Pharmaceutical Sciences Branch (IAUPS), Tehran, Iran.
P.O.Box: 193956466
Tel: +982122640051 Fax: +982122602059
E-mail address: awwadi@yahoo.com

1. Introduction

It has been known that fixed-dose combinations can improve medication compliance and result in better clinical outcomes in patients with chronic conditions (Sripal Bangalor et al., 2007). This therapy approach is recommended by treatment guidelines and is widely accepted by health care providers for the treatment of hypertension. Fixed-dose combinations provide an effective antihypertensive treatment by achieving normal Blood Pressure (BP) more frequently, improving patient compliance, and decreasing adverse side effects. Use of effective combination therapies blocks two or more BP regulatory systems and resulted in higher reduction in BP compared with most monotherapies (Eduardo Pimenta and Suzanne Oparil ., 2008) .

Lisinopril is chemically designated as (s)-1-N-[N-[(1S)-1-carboxy-3-phenylpropyl}-L-lysyl]-L-proline dehydrates. It is an inhibitor of angiotensin converting enzyme and is used as an antihypertensive (O'Neil JM et al., 2001) along with Hctz. Hctz, 6-chloro-1,1-dioxido-3,4-dihydro-2H-1,2,4- benzothiadiazine-7-sulfonamide, is a thiazide diuretic which reduces the reabsorption of electrolytes from the renal tubules. This leads to increased excretion of sodium and chloride ions, resulting in higher water excretion (Jain R and Jain C., 1991). The combination of Hctz and Lis is useful in treatment of mild to moderate hypertension. The commercial tablets contain 10 mg Lis and 12.5 mg Hctz..

The widespread use of these drugs in combination requires development of analytical methods for their simultaneous assay (N.V. Ramakrishna et al., 2005). Several analytical procedures have been proposed for the quantitative assay of Lis and Hctz separately. However, few methods are present in the literature for the simultaneous determination of Lis and Hctz. This paper describes a HPLC method for both the assay and comparative release studies of Hctz and Lis commercial tablets and our new design formulation.

2. Materials and Methods

2-1.Material and Equipment

2-1-1 Apparatus:

The High Performance liquid chromatograph (HPLC) Younglin Acme 9000 HPLC was used for assay studies and was equipped with a 210- nmc detector and an 4.6- mm × 20-cm column that contains 5μm packing L7. . The HPLC was performed at room temperature. Peak identity was confirmed by comparison of spectra and retention time with those of standard. Tablet hardness tester 5Y (Dr. Schleuniger Pharmaton-USA), was used to measure the hardness of tablets. Tablet friability tester FR1000 (Dr. Schleuniger Pharmaton-USA) was used to determine the friability of the tablets. Tablet disintegration tester DTG200 (Dr. Schleuniger Pharmaton-USA), was used to determine tablet time. Tablet dissolution tester USP DT60 (wka-Germany) was used to measure the release, and finally tablet compressing machine (Lorsch-Germany) was used to press the tablets.

2-1-2 Chemicals:

Generally, all chemicals used were the purest grade available and were used as received without further purification. Lisinopril was purchased from Lupin, India and Hydrochlorothiazide from Darupakhsh, Iran. Microcrystalline cellulose (Avicel PH 102). Mannitol, Pergelatinized starch, Magnesium Stearate, Polyvinylpyrolidon (PVPK30), Croscarmelluse Sodium (AcDiSol), Calcium phosphate dibasic, Talc and Starch were provided by Hakim Pharmaceutical Company. The water used was distilled and deionized. The Innovator tablets 1A-Pharma (Germany) were purchased locally.

2-1-3 Chromatographic conditions:

The mobile phase was consisted of water, acetonitrile, phosphoric acid, and triethyl-

amine with following ratios (1480:280:15:3). The mobile phase was always filtered using 0.45μm membrane filters. Buffer solution was prepared by dissolving 4.08 of mono basic potassium phosphate in 800 ml of water, the pH was adjusted with phosphoric acid to 2.5, diluted with water to a final volume of 1L and mixed well. The Buffer and sample solution were also filtered using 0.45μm membrane filters. The flow rate and run time were 1.5 ml/min and about 10 minutes, respectively.

2-1-4 Preparation of the standard solution:

Lisinopril Standard Preparation: An accurately weighted quantity of USP Lisinopril Reference Standard (RS) was dissolved in Buffer solution to obtain a solution with a known concentration of 0.5 mg/ml.

Hydrochlorothiazide Standard Preparation: An accurately weighted quantity of USP Hydrochlorothiazide RS was dissolved in methanol to obtain a solution having a known concentration of 3.12 mg/ml.

Combined Standard Preparation for dissolution test: An appropriate aliquot of Lisinopril and Hydrochlorothiazide standard solutions were transferred to a suitable volumetric flask and diluted with medium to obtain a solution containing 0.1mg/ml Lisinopril and 0.013 mg/ml Hydrochlorothiazide.

2-2 Methods

2-2-1 Preparation of tablets containing Lisinopril (10mg)-Hydrochlorothiazide (12.5mg) by wet granulation method:

Different formulations were designed by wet granulation to obtain the best formulation with acceptable physiochemical characteristics. Dissolution, assay and stability studies were carried out for all formulations and the results were compared with those of 1A-Pharma tablets. The composition of all prepared formulations is summarized in Table1. The active ingredients and excipients were carefully weighted. Active ingredients and a half of the disintegrator were mixed well. The binder solution was added to make a wet mass. The wet mass was passed through a 14 mesh sieve; the granules were dried in an oven for 15 min at 45ºC and then blended with lubricant and the remaining of the disintegrating agent. Tablets were compressed using 8mm diameter flat- faced punch at a final weight of 150 mg.

Table 1. Percentage components of the formulation designed by wet granulation method

Stearic Acid	Soudim Benzoate	Talc	Mgst	Pergelatinze Starch	Acdisol	Starch	PVP K_{30}	Ca Phosphat Dibasic	Mannitol	Avicel	HCTZ	Lis	Component %
_	_	_	1.000	_	2.000	_	4.500	_	_	77.490	8.340	6.670	WG_1
_	_	_	1.000	_	2.000	_	4.500	77.490	_	_	8.340	6.670	WG_2
_	_	_	1.000	_	2.000	_	4.500	_	77.490	_	8.340	6.670	WG_3
_	_	_	1.000	_	2.000	_	4.500	_	51.660	25.830	8.340	6.670	WG_4
_	_	_	1.000	_	2.000	_	4.500	_	38.750	38.750	8.340	6.670	WG_5
_	_	_	1.000	_	2.000	_	4.500	_	25.830	51.660	8.340	6.670	WG_6
_	_	_	1.000	_	2.000	10.000	_	_	_	71.990	8.340	6.670	WG_7
_	_	_	1.000	_	2.000	15.000	_	_	_	67.000	8.340	6.670	WG_8
_	_	2.500	_	_	2.000	_	4.500	_	38.000	38.000	8.340	6.670	WG_9
_	_	_	1.000	5.000	_	_	4.500	_	37.245	37.245	8.340	6.670	WG_{10}
_	_	_	1.000	5.000	_	_	3.500	_	37.750	37.750	8.340	6.670	WG_{11}
1.500	_	_	_	5.000	_	_	4.500	_	37.000	37.000	8.340	6.670	WG_{12}
_	2.500	_	_	5.000	_	_	4.500	_	36.500	36.500	8.340	6.670	WG_{13}
_	_	_	1.000	5.000	_	_	4.500	_	37.245	37.245	8.340	6.670	WG_{14}

2-2-2 Characterization studies:

The prepared tablets were characterized by physicochemical characteristics such as average weight, hardness, and friability and disintegration time. Dissolution test was performed for those formulations that passed the physicochemical tests. Assay and stability tests were performed for the best formulation and the results were compared with 1A-Pharma tablets. The results of physicochemical tests are summarized in (Table 2).

Table 2. The result of physicochemical tests of wet granulation formulation

Formulation	(mg) Weight variation	Disintegration (time (min)	(%)Friability	(Kpas) Hardness
	n=20	n=6	n=10	n=6
WG1	151.48 ± 3.5	0.46 ± 0.03	0.19	5.92 ± 0.66
WG2	151.47 ± 2.0	0.46 ± 0.13	0.72	3.65 ± 0.83
WG3	152.96 ± 2.8	1.21 ± 0.06	0.38	4.19 ± 0. 74
WG4	148.40 ± 3.6	1.20 ± 0.13	0.07	4.81 ± 0.40
WG5	150.37 ± 2.3	2.06 ± 0.29	0.26	6.62 ± 1.09
WG6	155.00 ± 2.6	1.03 ± 0.13	0.80	3.87 ± 1.34
WG7	151.20 ± 2.3	0.17 ± 0.02	0.27	6.57 ± 1.06
WG8	157.50 ± 3.5	0.32 ± 0.03	0.70	7.20 ± 0.43
WG9	152.22 ± 2.2	1.00 ± 0.02	0.40	5.52 ± 0.78
WG10	147.91 ± 3.6	0.95 ± 0.07	0.40	6.52 ± 0.91
WG11	145.50 ± 4.1	0.78 ± 0.12	0.40	4.65 ± 0.70
WG12	153.62 ± 3.2	3.87± 0.29	0.30	7.20 ± 1.02
WG13	-	-	1.67	6.28 ± 1.04
WG14	150.84 ± 1.1	3.04 ± 0.23	0.30	6.03 ± 0.48

2-2-3 Dissolution studies:

Dissolution studies on tablets were performed according to U.S. Pharmacopeia (USP, 2009) method, apparatus 2, test 1. Best formulated tablets and 1A-Pharma tablets were transferred into dissolution medium (900ml of 0.1N HCL). 10ml of samples were withdrawn at 5, 15, 30 and 45 minutes. The samples were filtered and analyzed by HPLC. Withdrawn samples were replaced by equal volumes of fresh dissolution medium. Separately, an equal volume (20μl) of working standard solution and test solution were injected into the chromatograph and the amount of dissolved Lisinopril and Hydrochlorothiazide were determined. The comparative release profiles of Lisinopril and Hydrochlorothiazide were presented in Figs 10 and 11, respectively.

2-2-4 Assay studies:

The percentages of the Lis and Hctz in different formulations were analyzed by HPLC method. To prepare assay stock, 10 tablets were transferred to a suitable volumetric flask and the Buffer solution (0.25 mg/ml of total Lisinopril) and methanol (0.5 mg/ml of total Hydrochlorothiazide) were added. The mixture was diluted with water to volume to obtain a solution with a known concentration of 0.4 mg/ml Lisinopril and 0.5 mg/ml Hydrochlorothiazide for tablet strengths of 10/12.5.

The assay stock was diluted with Buffer solution to obtain a solution with 0.1 mg/ml Lisinopril and 0.12 mg/ml of Hydrochlorothiazide for tablets.

A known volume (10 μl) of combined standard preparation and the assay preparation were separately injected into the chromatograph and the response for major peak and the percentage of Lisinopril ($C_{21}H_{31}N_3O_5$) and Hydrochlorothiazide ($C_7H_8ClN_3O_4S_2$) were calculated.

2-2-5 Stability Studies:

According to ICH protocol, an accelerated stability study on the best formulation was performed at 40±2ºC and 75±5 % humidity. The samples were analyzed for assay and release profile at 0, 1, 3 months.

3. Results and Discussion

3-1. Physicochemical characteristics

The physical characteristics such as average weight, hardness, and friability and disintegration time of fourteen groups of tablets prepared by wet granulation method are presented in Table 2. As presented, four optimized formulations (WG3, WG5, WG12, and WG14) were chosen for dissolution studies. The parameters for selection of the formulations were: 1) compressibility and hardness at the range of 4-8 Kpas, 2) Friability < 0.5%, 3) disintegration time > 1min and <15min and 4) weight variation of 150±7.5 mg according to USP guideline. Formulations WG1-WG6 were prepared to choose the best filler for formulation design. Formulation WG1 didn't pass the disintegration time test; data showed that the disintegration time for WG1 formulation was close to the minimum range of guideline and it seemed to have some problem in stability tests. Formulations WG2 and WG6 were rejected due to unsuitable friability test results (Table 2). As a result, the combination of Avicel and Mannitol 1:1 showed the best physicochemical results in friability, compressibility and physicochemical tests. Formulations WG3 and WG5 were chosen for dissolution analysis tests. In other formulations, the importance of binder role was analyzed and therefore PVP and corn starch were used. Corn Starch was used in formulation WG7 and WG8 as the binder. Although the compressibility of both formulations was acceptable, the disintegration time was too low to coat the tablets in pan coating. Other changes were the type and percentages of lubricant and disintegrant. The corresponding results are shown in formulations WG9, W10, WG11, WG12 and WG13. Formulation WG9 had the minimum range of disintegration, which may induce problem during scale up process. Formulation WG11 had a minimum range of compressibility and may damage tablets during packaging and storage. Formulation WG13 had an acceptable range of hardness; however, its disintegration time was too low and its friability fell out of acceptable range resulting in capping. Formulation WG12 has successfully passed all the physicochemical tests and was chosen as a candidate for further dissolution analysis test. In formulation WG14, the entire disintegrating agent was added at the end and showed acceptable physicochemical characteristics. This formulation was also chosen for dissolution studies along with formulations

WG3, WG5 and WG12.

3-2 Dissolution studies:

Dissolution studies were carried out on WG3, WG5, WG12, and WG14 to compare the percentage of drug release with 1A-Pharma tablets. Differential and similarity factors (F1<15, F2>50) were comparative parameter to choose our best formulation. Table 3 represents the dissolution results. As indicated, formulation WG14 successfully passed the F1, F2 rules, thus WG14 was chosen for

Table 3. Comparison of the release of the selected formulation with 1A-Pharma tablet release (F1:Similarity; F2: difference)

Formulation	Factor	Lisinopril	Hydrochlorothiazide
WG3	F1	14.86	10.65
	F2	39.52	53.55
WG5	F1	11.87	11.30
	F2	46.9	49.20
WG12	F1	24.8	33.80
	F2	26.1	27.40
WG14	F1	9.24	5.08
	F2	51.5	66.31

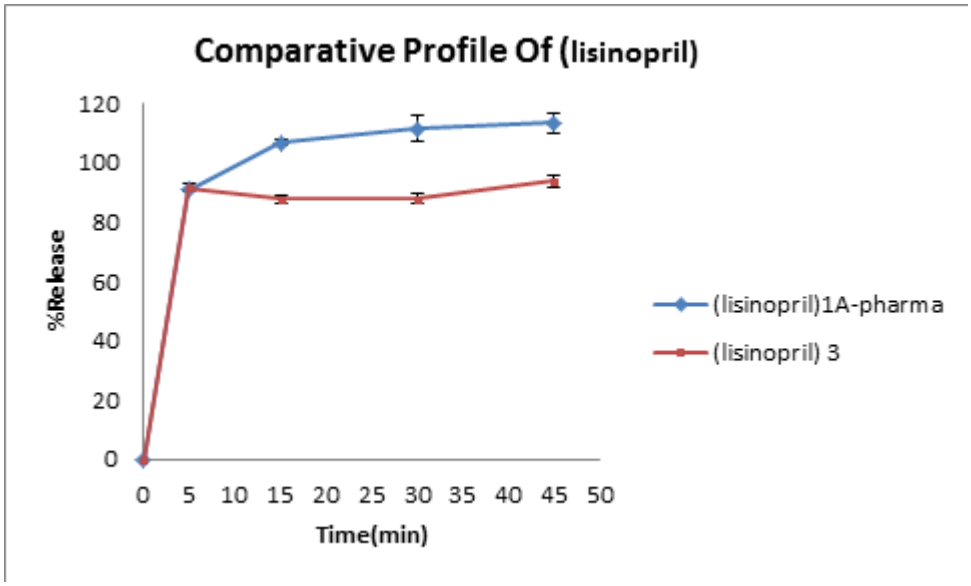

Figure 1. Comparative profile of Lis release from formulation WG3 and 1a-pharma (Germany) tablets

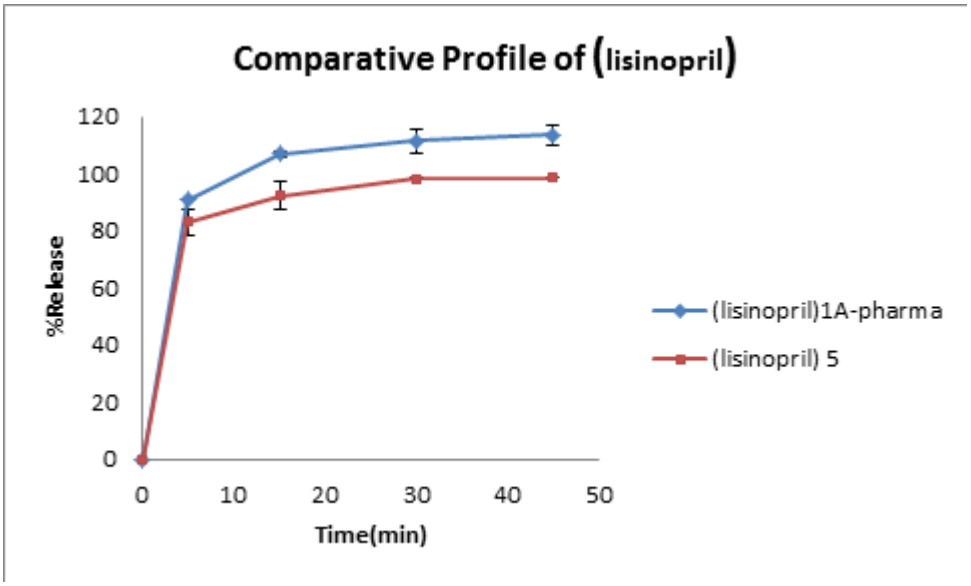

Figure 2. Comparative profile of Lis release from formulation WG5 and 1A-Pharma tablets

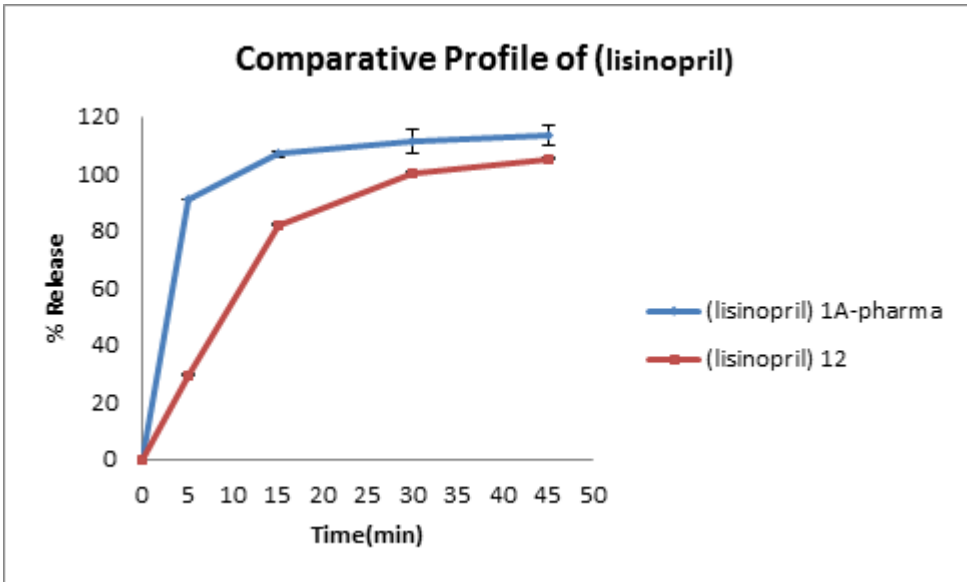

Figure 3. Comparative profile of Lis release from formulation WG12 and 1A-Pharma tablets

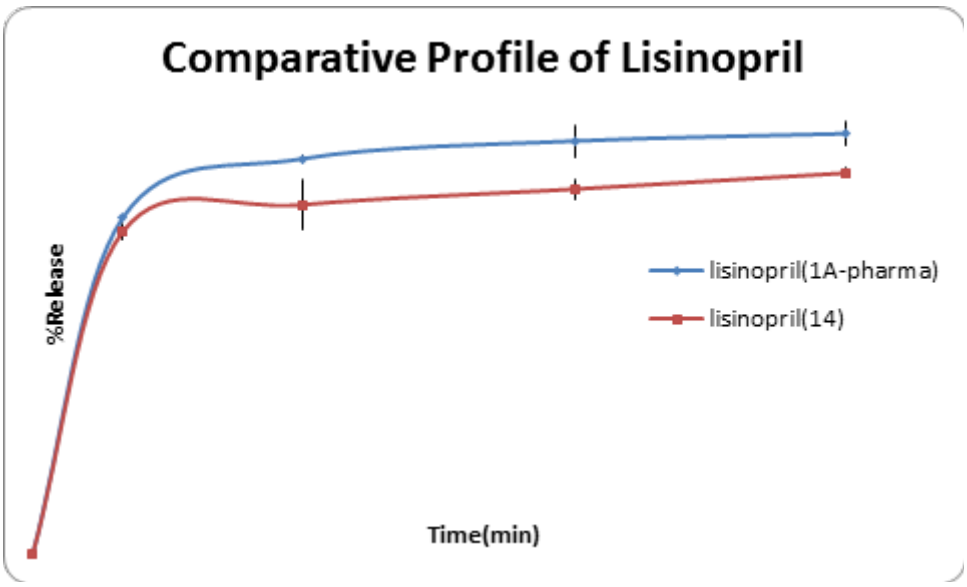

Figure 4. Comparative profile of Lis release from formulation WG14 and 1A-Pharma tablets

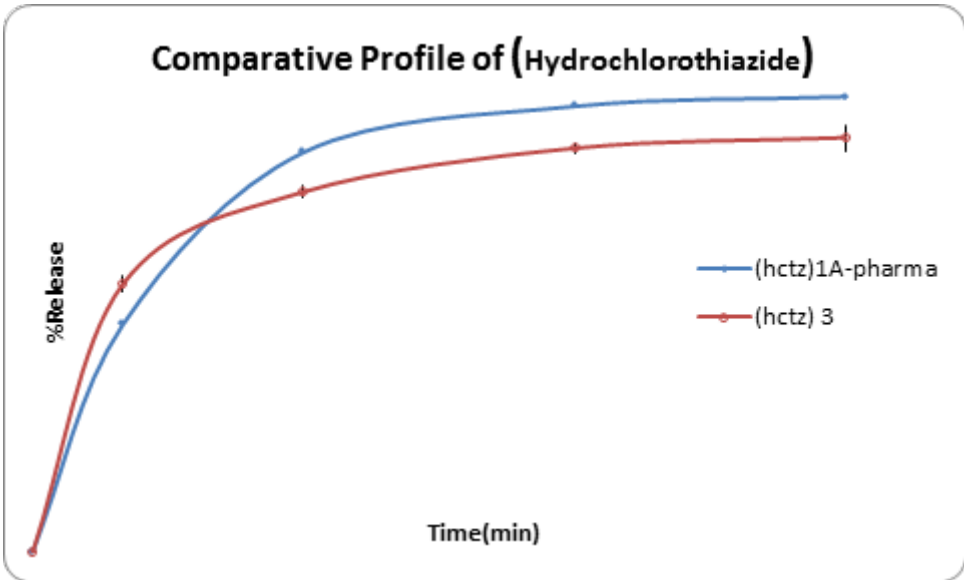

Figure 5. Comparative profile of Hctz release from formulation WG3 and 1A-Pharma tablets

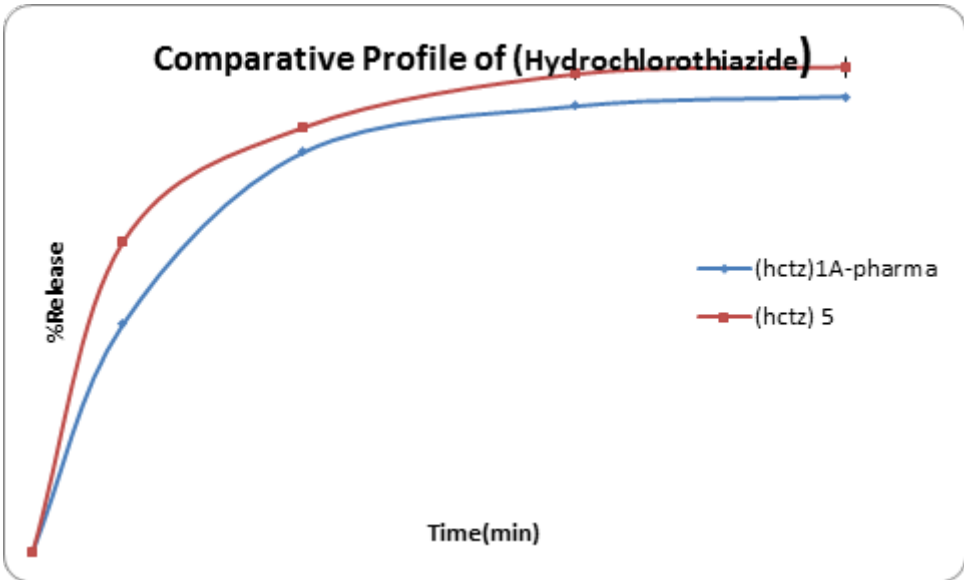

Figure 6. Comparative profile of Hctz release from formulation WG5 and 1A-Pharma tablets

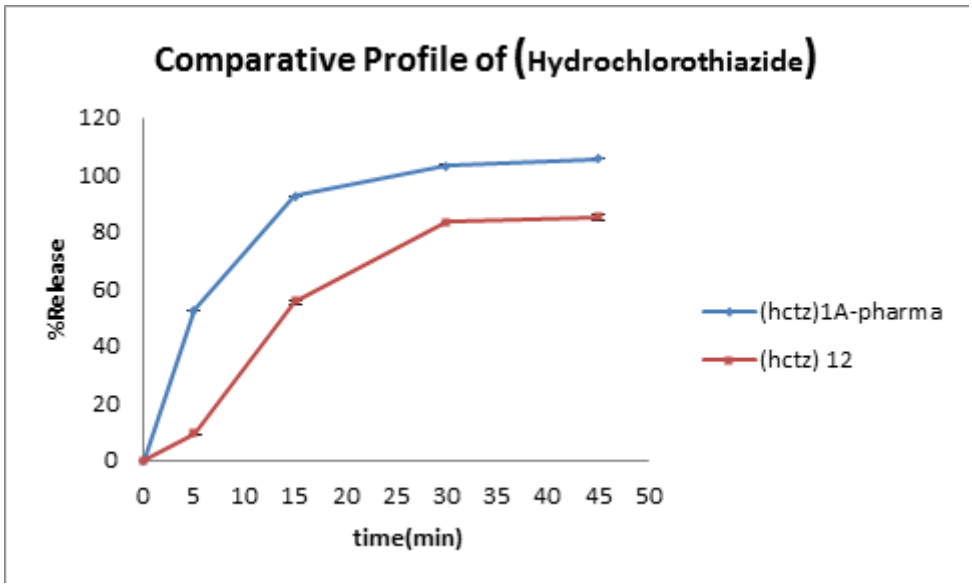

Figure 7. Comparative profile of Hctz release from formulation WG12 and 1A-Pharma tablets

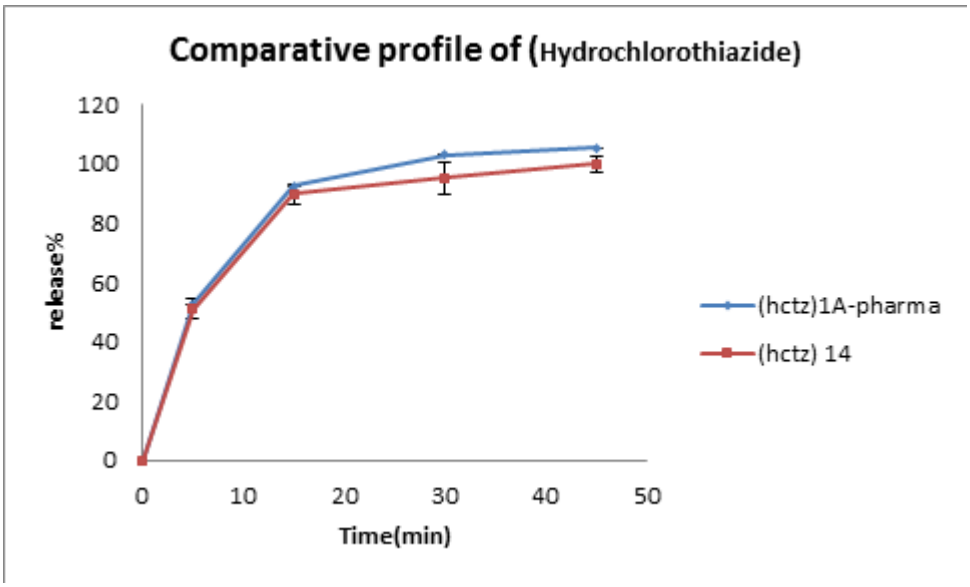

Figure 8. Comparative profile of Hctz release from formulation WG14 and 1A-Pharma tablets

further assay and stability studies. Figures 1-4 show the comparative release profile of Lis and Figs 5-8 illustrate the comparative release profile of Hctz from the selected formulation as compared with 1A-Pharma tablets.

3-3 Assay Studies

According to USP32 Lis and Hctz tablets contained not less than 90% and not more than 110% of the labeled amount of Lis and Hctz. Assay studies were carried out on 1A-Pharma and WG14 tablets and the results showed acceptable drug contents for WG14. (Lis:

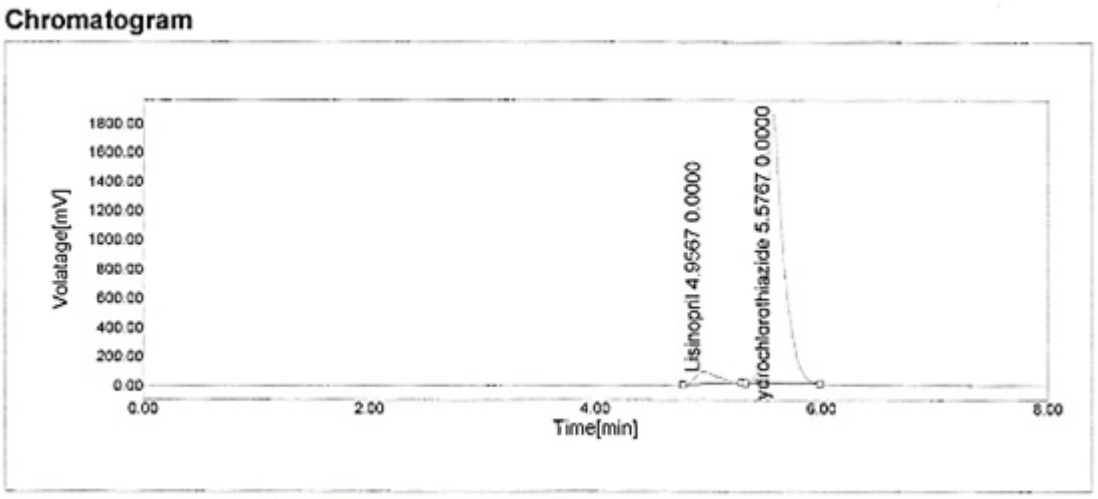

Figure 9. The HPLC peaks and retention times for Lisinopril and Hydrochlorothiazide.

100.47% and Hctz: 100.14%). The chromatogram obtained from HPLC is shown in Figure 9. As indicated in the figure, the retention time for Lis and Hctz is 4.95 and 5.58 minutes, respectively.

3-4 Stability Studies:

The samples were stored at 40 ± 2° C and 75±5% humidity showed no significant changes in the drug content or the release profile of either Hctz or Lis. However, both drugs contained not less than 80% of labeled amount of Lis dissolved in 30 min and not less than 80% Hctz dissolved in 45min.

Table 4. The result of Lisinopril assay test of formulation WG14 during accelerated stability test

Time of sampling / Active ingredient	Onset time	First month	Third month
Lisinopril	102.63	100.94	102.28

Table 5. The result of Hctz assay test of formulation WG14 during accelerated stability test

Time of sampling / Active ingredient	Onset month	First month	Third month
Hydrochlorothiazide	103.785	102.310	102.524

Accordingly, WG14 showed an acceptable range of drug content and showed no changes during the accelerated stability conditions. Hence, WG14 can be considered as the best formulation. Tables 4 and 5 illustrate the drug content of WG14 during accelerated stability test for Lis and Hctz. After accelerated stability tests, dissolution tests were done and the results were satisfactory and according to the guidelines. Accordingly, both drugs in formulation WG14 passed the tests. In addition, more than 80% of Lis and Hctz were dissolved within 30 and 45 minutes, respectively. Tables 6 and 7 represent the release percentages of drugs and Table 8 shows the results of F1, F2 after that the accelerated studies in third months. Figures 10 and 11 show the comparative released

Table 6. The time profile of Lis release from formulation WG14 during the accelerated stability studies conditions in third months

Time / Active ingredient	5min	15min	30min	45min
Percentage of released Lis in third month	86.47±0.50	90.20±0.64	92.44±0.51	95.47±0.61

Table 7. Percentage of released Hctz from formulation WG14 during accelerated stability studies in third month

Time / Active ingredient	5min	15min	30min	45min
Percentage of Hctz released in third months	56.23±4.13	62.47±0.47	76.94±1.79	86.57±1.36

Table 8. Comparison of the release profile of formulation WG14 during the accelerated stability condition in third month (F1: similarity; F2: difference)

Active ingredient	factor	Third month samples
lisinopril	F1	13.75
	F2	42.52
hydrochlorothiazide	F1	22.20
	F2	35.10

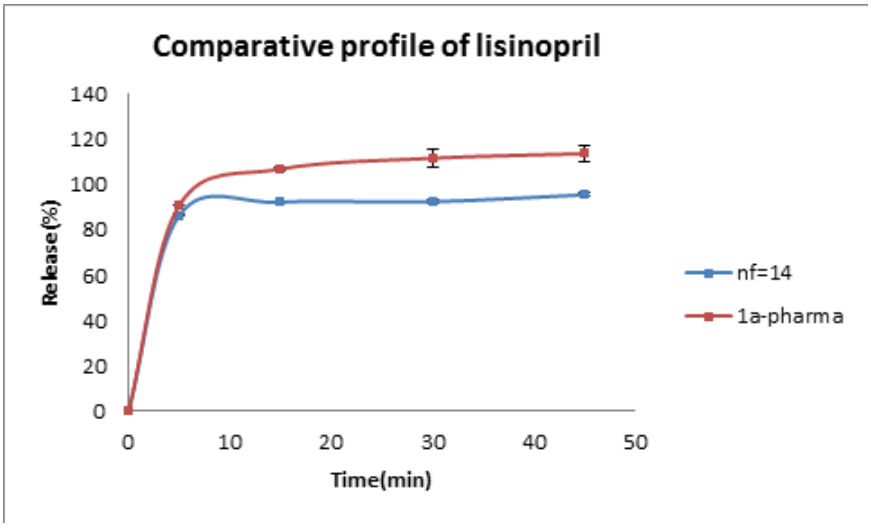

Figure 10. Comparative profile of Lis release from formulation WG14 and 1A-Pharma tablets after accelerated stability test (3rd month)

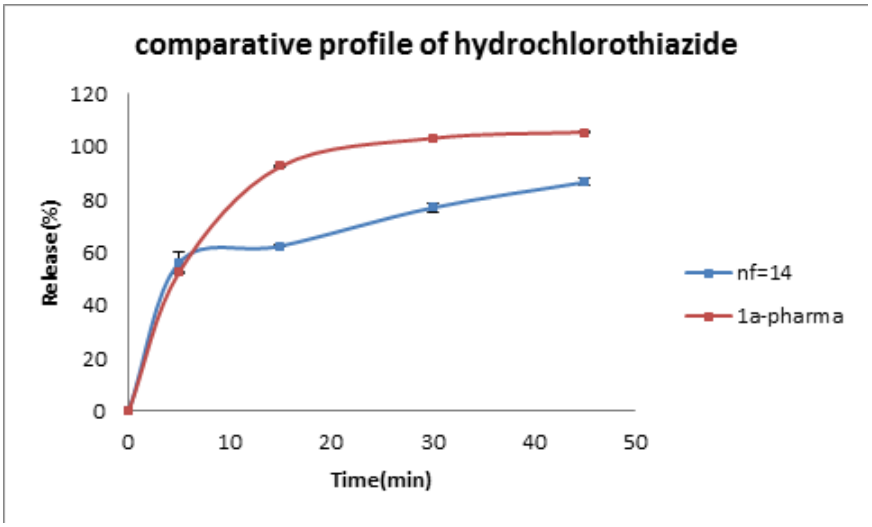

Figure 11. Comparative profile of Hctz release from formulation WG14 and 1A-Pharma tablets after accelerated stability test (3rd month).

profile of Lis and Hctz during the accelerated stability studies in third month. The data indicates that, this formulation successfully passed the tests and has the potential to be used in scale up process. The related long term stability studies will be pursued by Hakim Pharmaceutical Company.

4. Conclusion

High blood pressure (BP) is currently the major cause of morbidity and mortality world-wide (Ajay K Gupta et al., 2010). Combining blood-pressure-reducing drugs of the different classes is approximately 5 times more effective compared with simple doubling the dose of each drug (Ezzati M et al., 2002). Fixed dose combination alleviates medication risk, and improve patient outcome, hence it is considered as an effective treatment to chronic condition such as hypertension. In the present study, a new formulation for combination tablet of Lis10mg/Hctz 12.5mg was designed. Hctz and Lis tablets were prepared by wet granulation method. The formulation WG14 passed all physiochemical, dissolution, assay and stability test. The content of drugs did not change after 3 months during accelerated stability test. The release profiles of components were found acceptable as recommended by USP. We hope that production of this tablet in Iran will lead to economic opportunities and new treatment methods.

5. Acknowledgment

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