

Quantitative Analysis of Anti-Hypertensive drugs in Association (combination therapy) using IR and UV-Visible spectroscopy

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Abstract— This study presents the fast and simple method of identifying the physical mixture of antihypertensive drugs in association. It has been carried out to develop a novel and stable drug combination of Losartan and Hydrochlorothiazide. The spectroscopic techniques such as FTIR and UV-Visible have been employed to identify the bands of (pure) Hydrochlorothiazide and Losartan in that physical mixture. These methods have been carried out to develop a novel and stable drug combination of Losartan and Hydrochlorothiazide. The solid phase FTIR spectra of Losartan have been recorded in the region $4000\text{--}400\text{cm}^{-1}$. UV-Visible spectrum of the compound was recorded in the region $200\text{--}600\text{nm}$. The stable method of analyzing the antihypertensive drugs in association has been adopted which maintains the safety and potency of the active pharmaceutical ingredient present in the dosage form which adds value to the therapeutic agent.

Keywords— Hypertension, Losartan, Hydrochlorothiazide, combination therapy, FTIR, UV-Vis.

I. INTRODUCTION

Meticulous control of blood pressure is required in patients with hypertension to produce the maximum reduction in clinical cardiovascular end points [1]. Monotherapy is effective in achieving this target goal in only about 50 percent of patients. Treatment with two or more agents from different pharmacologic classes is often necessary to achieve adequate blood pressure control [2]. The rationale for using fixed-dose combination therapy is to obtain increased blood pressure control by employing two antihypertensive agents with different modes of action and to enhance compliance by using a single tablet that is taken once or twice daily [3]. Using low doses of two different agents can also minimize the clinical and metabolic effects that occur with maximal dosages of the individual components of the combined tablet [4]. These potential advantages are such that some investigators have recommended using combination antihypertensive therapy as

initial treatment, particularly in patients with target-organ damage or more severe initial levels of hypertension [5-8]. Different classes of antihypertensive agents which have been commonly used are angiotensin receptor blockers, thiazide diuretics, beta and alpha blockers, calcium antagonists and angiotensin converting enzyme inhibitors. The majority of currently available fixed-dose combinations are diuretic based [1] which are effective in reducing BP. A fixed dose combination of Losartan and Hydrochlorothiazide therapy may be a logical choice for antihypertensive treatment, including for initial therapy in patients with blood pressure elevation. Losartan Intervention for Endpoint reduction in hypertension (LIFE) study demonstrate that there was a 25% risk reduction for stroke in the administration of Losartan with Hydrochlorothiazide. The efficacy and degree of tolerability of this combination therapy may lower the risk for stroke in patients.

In the present investigation, the antihypertensive drugs such as Losartan (Angiotensin Receptor Blocker) and Hydrochlorothiazide (Diuretic) have been chosen for investigation. Losartan (LOS) is an effective antihypertensive drug. It belongs to a group of drugs called angiotensin II receptor antagonists. Losartan is chemically known as 2-butyl-4-chloro-1-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1H-imidazol-5-yl)methanol. Losartan is a nonpeptide molecule with the molecular formula $\text{C}_{22}\text{H}_{23}\text{ClN}_6\text{O}$. The molecular structure of Losartan is shown in Fig. 1.

In long-term trials, diuretics have been shown to reduce the incidence of stroke, congestive heart failure, coronary artery disease and total mortality from cardiovascular disease. Diuretics blunt the sodium and water retaining effects of many other antihypertensive drugs. So, they are the most commonly used medication in combination antihypertensive agents. Treatment with a diuretic such as hydrochlorothiazide results

in a dose-dependent blood pressure reduction that levels off with higher dosages [9]. The Joint National Committee (JNC) VI states that its addition will enhance the effects of other agents [10].

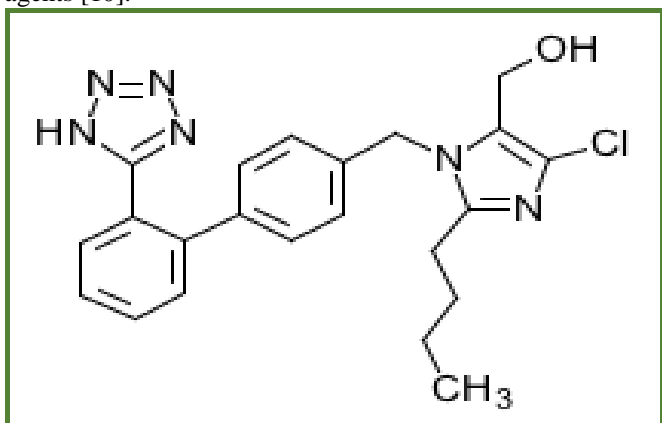


Fig. 1. Molecular structure of Losartan

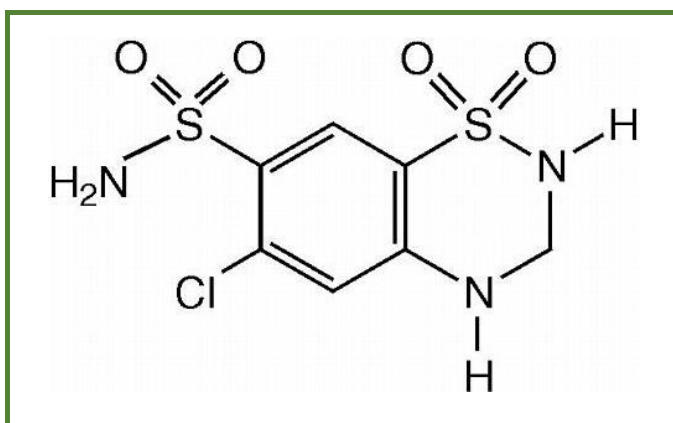


Fig. 2. Molecular structure of Hydrochlorothiazide

Hydrochlorothiazide (HCTZ, HCT or HZT) is the most commonly prescribed antihypertensive drug. It is a thiazide diuretic drug or waterpill, which prevents the body from absorbing excess salt that creates fluid retention. It is chemically known as 6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide with the molecular formula $C_7H_8ClN_3O_4S_2$. The molecular structure of Hydrochlorothiazide is shown in Fig. 2. The combination of angiotensin receptor blockers and Diuretics antihypertensive drugs are recommended to achieve BP goal [11]. It also permits the use of low doses and improves adherence to therapy. Moreover, the Pharmaceutical associations should involve only the physical mixture and not the interaction persists within them. The strength of interaction may influence the molecular arrangement of drugs. This may give rise to several changes in the solid state, like phase transition [12]. Interactions of drugs from their association may cause serious illness or sometimes lead to death. Luciano et al [13] had reported the association of hypertensive drugs and their identification using Raman spectroscopy. S. Karthikeyan [14] had reported the similar work in his paper. With that motivation, the present research has been carried out to

develop a novel drug combination of Losartan and Hydrochlorothiazide using IR and UV-Visible spectroscopy. As dosage form is the physical form of a dose of drugs, the chosen Losartan and Hydrochlorothiazide were mixed physically in various stoichiometric ratios. These physical mixtures were used as such for further investigation.

II. MATERIALS AND METHODS

The pure drugs namely Losartan and Hydrochlorothiazide were procured from Sigma-Aldrich Chemical Company, USA with a stated purity of greater than 98% and it was used as such without any further purification. These pure drugs were mixed physically in various stoichiometric ratios such as 1:1, 1:2, 2:1, 1:4 and 4:1. The combined pharmaceutical formulations in various stoichiometric ratios can be easily distinguished and characterized using spectral analysis. The spectroscopic techniques such as FTIR and UV-Visible have been employed to identify the bands of (pure) Hydrochlorothiazide and Losartan in that physical mixture. The FTIR spectrum has been recorded in the region $4000-400\text{cm}^{-1}$ using KBr pellet technique with 4cm^{-1} resolution on PERKIN ELMER SYSTEM ONE FTIR/ATR spectrometer at SAIF, IIT Madras, India. UV-Visible spectrum has been recorded in the region $200-600\text{nm}$ using Lambda35 at SAIF, St. Peter's University Chennai, India.

III. RESULTS AND DISCUSSIONS

A. FTIR Analysis in Drug Association

The combined pharmaceutical formulations in various stoichiometric ratios can be easily distinguished and characterized using spectral analysis. The IR spectra of Losartan and Hydrochlorothiazide associations in different proportions are shown in the Fig. 3. The presence of characteristic diuretic bands is to be identified, as they are used in the minimum dosage when compared with Losartan in the physical mixture. At 1:1 rate, the characteristic band at 1520cm^{-1} and 1460cm^{-1} were assigned to indicate the presence of Hydrochlorothiazide. The drugs were taken in the minimum dosage of 1mg of Losartan and 1mg of Hydrochlorothiazide.

As the quantity of HYD in the mixture is increased in 1:2 and 1:4 rates, some more characteristic bands of HYD appear at 1556cm^{-1} , 1374cm^{-1} and 1019cm^{-1} . At 1:4 rate, the spectrum contains all the characteristic bands of Hydrochlorothiazide are present and have relatively increase in the intensity of absorbance. This is due to the large quantity of HYD drug in the mixture.

From the IR spectrum of LOS and HYD association in the proportion 2:1 (2mg of Losartan and 1mg of Hydrochlorothiazide), the relative intensities of characteristic bands such as 2953 , 2688 and 726cm^{-1} were increased when compared with 1:1 proportion of drugs in association. This is due to the higher proportion of this drug in the mixture. The intensity of an absorption band depends on the change in the dipole moment of the bond and the number of specific bonds present. Additionally, the number of specific bond also

determines the intensity of a peak Moreover, when Losartan is

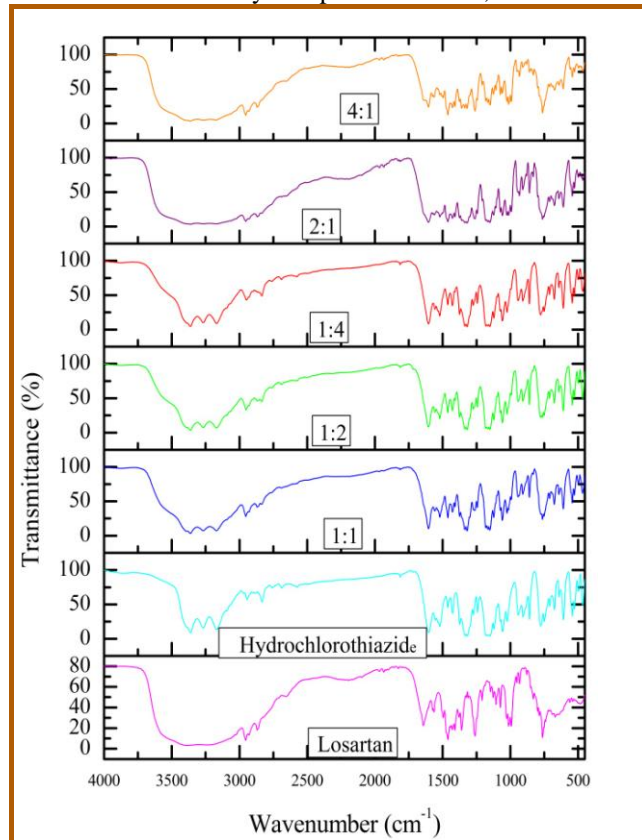


Fig. 3. FTIR Spectra of Losartan in association with Hydrochlorothiazide in different proportions

taken in the higher proportion like 4:1 rate, the bands of Losartan dominates in the spectrum. It is highly difficult to characterize the HYD (diuretic) bands due to the overlapping between the bands of these drugs in association.

Thus the characteristic peaks corresponding to the pure drugs Losartan and Hydrochlorothiazide were seen in the spectra of the physical mixture of these pharmaceutical associations in various stoichiometric ratios. The spectra showed no substantial shifting of the position of functional groups. The intensity of absorbance alone varies indicating no major interaction.

B. UV-Visible Analysis in Drug Association

In order to support the work done by FTIR spectroscopic technique, the UV-Visible analysis has been employed to identify characteristic bands of pure drugs in physical mixture. The UV-Vis spectrum of pure Losartan shows maximum absorption at 205nm whereas the Hydrochlorothiazide in its pure form show broad absorption spectrum having three wavelength maximum at 226, 272, 317nm with the absorbance values of 1.34, 0.710 and 0.122 respectively. In order to investigate the association of drugs, they are mixed in different stoichiometric ratios such as 1:1, 1:2, 2:1, 1:4 and 4:1. The UV-Vis spectrum of pure Losartan and Hydrochlorothiazide in various proportions and their corresponding absorbance values are listed in Table I.

TABLE I. VARIATION OF ABSORBANCE (ABS) OF LOS AND HYD IN VARIOUS PROPORTIONS

Drugs	λ_{\max}	Abs	λ_{\max}	Abs	λ_{\max}	Abs
LOS	205	1.62	-	-	-	-
HYD	226	1.34	272	0.710	317	0.122
1:1	224	2.65	270	1.08	317	0.150
1:2	225	2.37	272	1.09	317	0.172
1:4	225	1.98	272	0.93	317	0.146
2:1	211	1.70	269	0.64	317	0.83
4:1	207	1.90	-	-	-	-

In the case of 1:1 ratio of LOS/HYD (where 1mg of LOS and 1mg of HYD), the characteristic bands of Hydrochlorothiazide appear. A shift in the HYD was observed due to the association of LOS in the physical mixture. An increase in the absorbance was observed at the peaks 225nm (absorbance is 2.656), 270nm (absorbance 1.08) and 317nm (0.150). Fig. 4 shows the UV-Visible spectrum of LOS and HYD drugs in association.

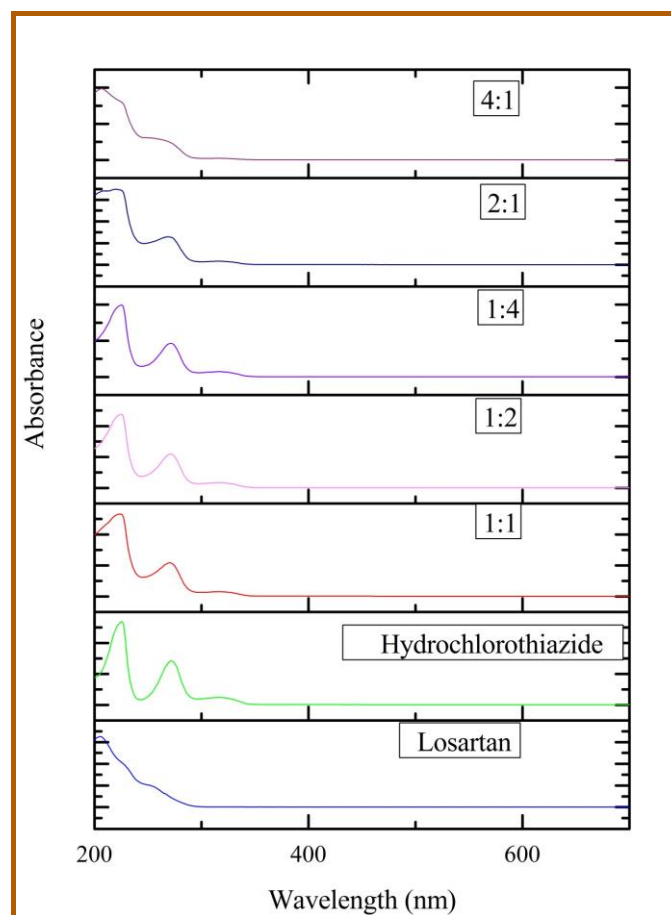


Fig. 4. UV- Vis Spectra of Losartan in association with Hydrochlorothiazide in different proportions

The quantity of Hydrochlorothiazide is increased at 1:2 (where 1mg of LOS and 2mg of HYD) and 1:4 ratios (where 1mg of LOS and 4mg of HYD). It was observed that increasing the concentration of HYD shows decrease in the

absorbance value which does not make any significant change in the λ_{max} . The bands of LOS are not present. This is due to the large quantity of HYD in that mixture. This confirms the association of Losartan and Hydrochlorothiazide in well manner. On the other hand, in the ratio 1:4, the profile of the spectrum is the characteristic of Hydrochlorothiazide drug.

At 2:1 ratio (where 2mg of LOS and 1mg of HYD), the absorbance value is decreased with the shift in the wavelength and in the ratio 4:1, it shows only one peak at 207nm. The spectrum depicts the profile of losartan due to the larger quantity of Losartan in that physical mixture.

IV. CONCLUSION

The combined pharmaceutical formulations in various stoichiometric ratios are distinguished and characterized using spectral analysis. This developed the stable method of analyzing the antihypertensive drugs in association has been adopted which maintains the safety and potency of the active pharmaceutical ingredient present in the dosage form which adds value to the therapeutic agent. The optimization of this fast and simple method of analyze is important mainly in antihypertensive drugs association.

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