Simultaneous Determination of Hydrochlorothiazide and Irbesartan from Pharmaceutical Dosage Forms with RP-HPLC

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INTRODUCTION: In this work, a simple and rapid liquid chromatographic method for the simultaneous determination of irbesartan (IRBE) and hydrochlorothiazide (HCT) was developed and validated using reverse phase high performance liquid chromatographic (RPLC) method.

METHODS: Experimental conditions such as; different buffer solutions, various pH values, temperature, composition of the mobile phase, the effect of flow rate were optimized.

RESULTS: The developed RPLC method for these antihypertensive agents was wholly validated and IRBE was detected in the linear range of 0.1-25 µg.mL^{-1} and HCT was detected in the linear range of 0.25-25 µg.mL^{-1}. Moreover, the suggested chromatographic technique was successfully applied for the determination of the drugs in pharmaceutical dosage forms with LOD values of value of 0.008 µg.mL^{-1} for IRBE and 0.012 µg.mL^{-1} for HCT.

DISCUSSION AND CONCLUSION: It is believed that the proposed rapid analyses method of these antihypertensive drugs can be easily used and applied in pharmaceutical companies where the analyses time is important.

Key words: HPLC, Irbesartan, Hydrochlorothiazide, pharmaceutical dosage forms

INTRODUCTION

Cardiovascular disease causes the death of 17.9 million people annually, which represents nearly 30% of all global deaths.[1] Hypertension is responsible for at least 45% of deaths due to heart disease. Variety of pharmaceuticals, such as angiotensin converting

enzyme inhibitors, angiotensin-receptor blockers, beta-blockers, diuretics and calcium channel blockers have been applied for the management of hypertension.[2]

Irbesartan (IRBE) (2-butyl-3-[p-(o-1 H-tetrazol-5-yl phenyl)benzyl]-1,3-diazospiro[4,4]non-1-en-4-one), is an angiotensin II receptor antagonist, which selectively and noncompetitively binds to the angiotensin II receptor subtype I (Figure 1). It has no affinity for the all receptor subtype 2, or α1- and α2-adrenoceptor and serotonergic receptors.[3–6] IRBE is mainly used for the treatment of hypertension. Furthermore, it may also plays roles in postponement of progression of diabetic nephropathy. It has also a role in the indication for the reduction of renal disease progression in patients with type 2 diabetes, hypertension, and microalbuminuria or proteinuria.[3–6] Hydrochlorothiazide (HCT), 6-Chloro-1,1-dioxo3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide, is one of the oldest member of thiazide class of diuretics (Figure 1). Thiazides decrease peripheral resistance by an unknown mechanism and thereby lower blood pressure. HCT is widely used in the form of combination pill with other antihypertensive agents including IRBE. [7–9]

Figure 1 here

Combined cardiovascular therapy is beneficial, since most of these therapies exploit complementary mechanisms of different acting antihypertensive agents to maximize blood pressure-lowering effects.[10] In the literature several methods have been described concerning simultaneous determination of IRBE and HCT in pharmaceutical dosage and biological samples using spectrophotometric[11–13] spectrofluorometric[14], capillary electrophoretic[15] and chromatographic methods.[16,17]

In this work, liquid chromatographic method for the simultaneous determination of IRBE and HCT was developed using RP-HPLC. Experimental conditions such as; different buffer solutions, various pH values, temperature, additives, the effect of flow rate were

optimized. Moreover, the developed chromatographic technique was successfully applied for the determination of IRBE and HCT in pharmaceutical dosage forms.

EXPERIMENTAL

Reagents and Chemicals

Irbesartan and hydrochlorothiazide their dosage forms Karvezide® and Co-Irda® were obtained from pharmaceutical companies. All reagents were in analytical grade and were prepared by doubly distilled water. All experiments were performed at room temperature; all solutions were protected from light and used within 24 h to avoid decomposition. Chromatographic grade acetonitrile, methanol, hydrogen peroxide, phosphoric acid were used. All other reagents were in analytical grade.

Instrumentation

Analyses were performed with the Agilent Technologies HP 1100 series (Wilmington DE, USA) LC system equipped with a G1379A degasser, G1311A quaternary pump, 61313 auto injector and G1315B diode array detector. Chromatographic separation was achieved using 60:40 (V/V)ACN:pH 3 phosphate buffer (from H_3PO_4) as a mobile phase, using an X Terra RP® (250 x 5 mm I.D.;3 µm) column at a flow rate of 0.8 mL min^{-1}, injection value of 10 µL. The separation was carried out at 30°C and the diode array detector adjusted to 226 nm. Before using the mobile phase, the pH was adjusted with 5M NaOH, and the mobile phase filtered through 0.45 µm Polytetrafluoroethylene membranes using with a vacuum pump and degassed. For the pH measurements, a pH meter Model 538 (WTW, Weilheim, Germany) was used with a combined electrode an accuracy of ± pH.

Preparation of Stock Solutions

Stock solutions of IRBE and HCT (100 µg.mL^{-1}) were prepared by dissolving 5 mg of each compound in 50 mL methanol by ultrasonication 10 min in ultrasonic bath. The required concentration for the analysis was diluted with the mobile phase.

Analysis of Pharmaceutical Dosage Form

For both binary mixtures, 10 tablets of Karvezide® and Co-Irda® were accurately weighed, crushed and finely powdered, separately. From these powders, desired solutions were prepared, sonicated and filtered. The analyzed solutions were obtained by diluting with mobile phase. The content of IRBE and HCT were calculated from the related calibration curves.

System suitability test studies and validation of the analytical method

The system suitability test parameters such as retention time, symmetry factor, theoretical plate number, selectivity, resolution, and tailing were calculated and compared with the recommended values in USP criteria's.[18,19,20] Validation of the proposed method was utilized according to ICH Guidelines and USP criteria in terms of precision, accuracy, linear range, limit of detection and quantification values and reported.

Degradation Studies

It is recommended by ICH Guidelines to perform degradation studies for the developed assays to show the method is stability indicating.[21,22] In order to assess the capacity of the proposed method to separate IRBE and HCT from their degradation products, mild and hard conditions like heating in oven (75°C, 3 h and 24 h), treating the samples with acidic (0.5 N HCl and 1 N HCl), alkaline (0.5 N NaOH and 1 N NaOH) solutions, or oxidants (3% and 30% H_2O_2), and exposing the UV (254 nm, 3 h and 24 h) were applied.

RESULTS AND DISCUSSIONS

System suitability test parameters

Prior to the validation of the proposed method, system suitability tests such as tailing factor, selectivity, resolution, tailing, theoretical number of plates were calculated reported. The retention factor values IRBE and HCT were calculated and found as 2.08 for HCT and 3.35 for IRBE, other system suitability test parameters are summarized in table 1. It was reported in Table 1, that the proposed method for the determination of IRBE and HCT was suitable related with ICH guidelines[21].

Table 1 about here

Method Validation

Linearity: Linearity of the detector for IRBE and HCT was evaluated by the relation between the peak areas under the corresponding peaks and concentrations of each drug with a correlation coefficient of 0.997 and 0.998, respectively (Table 2). The repeated injections were performed at each concentration levels. The linear range is in good agreement and have wide linear range values were obtained. For HCT, linear range was obtained between 0.25 and 25 $\mu g.mL^{-1}$ and for IRBE linear range was obtained between 0.1 and 25 $\mu g.mL^{-1}$ (Figure 2)

Table 2 here

Figure 2 about here

Limit of Detection and Limit of Quantification: ICH guidelines proposed several approaches for the determination of limit of detection (LOD) and limit of quantification (LOQ). In this work limit of detection (LOD) and limit of quantification (LOQ) values were calculated related with the relation LOD= 3.3 s/m and LOQ = 10 s/m using the standard deviation of response (s) and the slope (m) of the calibration curve. [19,22–24] LOD, LOQ values were also reported in Table 2, where statistical evaluation of the calibration data were presented. HCT was detected with LOD value of 0.012 $\mu g.mL^{-1}$ and LOQ value of 0.036 $\mu g.mL^{-1}$ with the

propped RP-HPLC method. IRBE was detected with LOD value of 0.008 $\mu g.mL^{-1}$ and LOQ value of 0.023 $\mu g.mL^{-1}$ with the propped RP-HPLC method.

Precision: Precision of the proposed method was evaluated by the repeatability studies. Within day and between day repeatability were determined by the injection of three different levels of calibration solutions (n=5) on the same day and three consecutive days, respectively. Results are expressed by the percentage of relative standard deviation (RSD%) and shown in Table 2, where statistical evaluation of the calibration data were presented. As seen, there is no significant difference between the values within the within day and between day measurements in mobile phase.

Accuracy: Accuracy is one of the important parameters for the analysis of pharmaceuticals. Real sample applications and recovery studies were performed to show the accuracy of the purposed method. Acceptable results were obtained for Karvezide® and Co-Irda® which contains 150 mg IRBE and 12.5 mg of HCT (Table 3). For the accuracy test, recovery of the method was studied using spiking method. Solutions were prepared by adding 50%, 100% and 150% levels standard solutions of the drugs to the pharmaceutical Karvezide® sample (Table 4). The experiment was performed in triplicate and recovery%, RSD%, and BIAS% spiked drugs were calculated. It can be resulted that, using the suggested HPLC method, acceptable recoveries can be obtained with RSD% values lower than 1 and recovery values between 98-102%.

Table 3 about here

Table 4 about here

Specificity (degradation studies)*:* Selectivity and specificity is another essential parameter in pharmaceuticals analysis[19,21–23]. Specificity studies were performed by means of degradation studies. Degradation of drugs under mild and drastic stress conditions were given in Table 5, as degradation %. It can be resulted that, the hard acidic and alkaline conditions hardly affect

these drugs. In UV and heat degradation these drug can be affected within 3 h already (Table 5).

Table 5 about here

CONCLUSION

In the present work, an RP- HPLC method was developed and validated for the simultaneous separation and determination of Irbesartan and Hydrochlorothiazide. The studies were performed for the analyses of Irbesartan and Hydrochlorothiazide from mobile phase. Compared to already published papers we suggest environmentally friendly, green chemistry method for the application to combined drug technology. It is believed that the proposed rapid analyses method of these antihypertensive drugs can be easily used and applied in pharmaceutical companies where the analyses time is important. Moreover, in studies with real samples, or in bioequivalence studies this method may be used.

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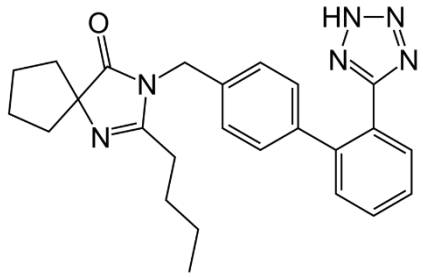
Figure 1. Chemical Structures of A) Irbesartan and B) Hydrochlorothiazide

A



B

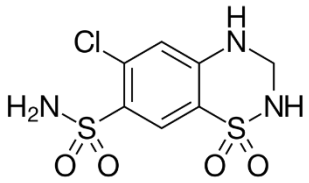


Figure 2. Chromatograms of increasing concentrations of Hydrochlorothiazide and Irbesartan

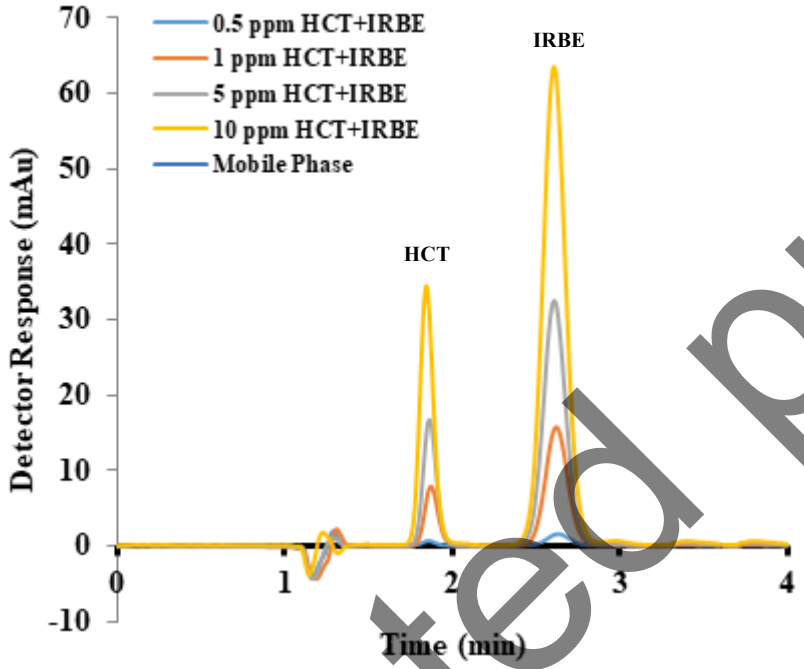


Table 1. System Suitability Tests Parameters

Parameters			Recommended Values[20]
Compounds	**HCT**	**IRBE**	
Retention Time (min)	1.85	2.61	
Selectivity	-	2.05	>1
Resolution	-	2.03	>2
Tailing	1.18	1.20	<2
Theoretical number of plates	4581	5402	>2000

Table 2. Statistical evaluation of the calibration data

	Mobile Phase	
Compounds	**HCT**	**IRBE**
Retention time(min)	1.85	2.61
Linearity range (µg.mL^{-1})	0.25-25	0.1-25
Slope (mAU.µg^{-1}.mL)	7.925	37.913
Intercept (mAU)	-0.313	9.438
Correlation coefficient	0.997	0.998
LOD (µg.mL^{-1})	0.012	0.008
LOQ (µg.mL^{-1})	0.036	0.023
Within day Repeatability [a] (RSD %)	0.775	0.265
Between day Repeatability [a] (RSD %)	0.848	0.430

Table 3. Results of tablet analysis from Karvezide® and Co-Irda®

	HCT	IRBE
Label Claimed (Karvezide® mg)	150.00	12.5
Found [a] (mg)	150.59	12.42
RSD (%)	0.20	0.11
Bias (%)	-0.39	0.63
Label Claimed (Co-Irda® mg)	150	12.5
Found [a] (mg)	149.72	12.45
RSD (%)	0.71	0.19
Bias (%)	0.18	0.37

[a] Each value is the mean of five experiment.

Table 4. Results of analysis from pharmaceutical dosage form Karvezide®

	50 % Accuracy		100 % Accuracy		150 % Accuracy	
	HCT	**IRBE**	**HCT**	**IRBE**	**HCT**	**IRBE**
Added (mg)	5.00	5.00	10.00	10.00	15.00	15.00
Found[a] (mg)	5.10	4.98	10.02	10.32	14.95	14.97
Recovery (%)	102.07	99.73	100.16	100.33	99.64	99.81
RSD (%)	0.19	0.25	0.51	0.18	0.85	0.15
Bias (%)	-2.07	0.26	-0.16	-0.33	0.36	0.19

[a] Each value is the mean of five experiment.

Table 5. Results of stress conditions by RP-LC in terms of degradation%

	Conditions	HCT	IRBE
mild conditions	**HCl (0.5 M)**	43.97	77.10
	NaOH (0.5 M)	33.16	25.15
	H_2O_2 (3 %)	12.16	84.49
	UV light exposure (3h at 254 nm)	90.18	90.98
	Oven (3h at 75°C)	95.11	72.56
hard conditions	**HCl (1 M)**	49.98	79.69
	NaOH (1 M)	37.23	59.95
	H_2O_2 (30 %)	25.21	93.91
	UV light exposure (24h at 254 nm)	97.48	78.46
	Oven (24h at 75°C)	96.97	87.87

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