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ORIGINAL ARTICLE

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

Farmasötik Dozaj Formlarında TF-YPSK ile Hidroklorotiyazid ve İrbesartanın Eş Zamanlı Tayini

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ABSTRACT

Objectives: In this work, a simple and rapid liquid chromatographic method for the simultaneous determination of irbesartan (IRBE) and hydrochlorothiazide (HCT) was developed and validated by reverse phase high performance liquid chromatography (RP-HPLC).

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

Results: The developed RP-HPLC 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 human serum and pharmaceutical dosage forms with limit of detection values of 0.008 μg mL^{-1} for IRBE and 0.012 μg mL^{-1} for HCT.

Conclusion: The proposed rapid analysis method of these antihypertensive drugs can be easily used and applied by pharmaceutical companies for which the analysis time is important.

Key words: HPLC, irbesartan, hydrochlorothiazide, pharmaceutical dosage forms

ÖZ

Amaç: Bu çalışmada, irbesartan (IRBE) ve hidroklorotiyazidin (HCT) eşzamanlı tayini için basit ve hızlı bir ters fazlı yüksek performanslı sıvı kromatografisi (TF-YPSK) yöntemi geliştirilmiş ve validasyon çalışmaları yapılmıştır.

Gereç ve Yöntemler: Deneysel koşullar; farklı tampon çözeltileri, çeşitli pH değerleri, sıcaklık, mobil fazın bileşimi, akış hızının etkisi gibi parametrelerin üzerinden optimize edildi.

Bulgular: Bu antihipertansif ajanlar için geliştirilen TF-YPSK yönteminin tüm validasyon parametrelerine ilişkin çalışmalar yapılmış, ve IRBE 0,1-25 μg mL^{-1} doğrusal aralığında ve HCT 0,25-25 μg mL^{-1} doğrusal aralığında tespit edilmiştir. Ayrıca önerilen TF-YPSK yöntemi ile IRBE için 0,008 μg mL^{-1} ve HCT için 0,012 μg mL^{-1} tayin alt sınır değerleri bulunmuştur. Geliştirilen yöntem, insan serumunda ve farmasötik dozaj formlarında bulunan IRBE ve HCT'nin belirlenmesi için başarıyla uygulanmıştır.

Sonuç: Bu antihipertansif ilaçların miktar tayininde önerilen YPSK analiz yönteminin, analiz süresinin önemli olduğu ilaç firmalarında rahatlıkla kullanılabileceği ve uygulanabileceği düşünülmektedir.

Anahtar kelimeler: YPSK, irbesartan, hidroklorotiyazit, farmasötik dozaj formları

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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. A 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 that selectively and noncompetitively binds to the angiotensin II receptor subtype I (Figure 1). It has no affinity for the 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 play roles in postponement of the progression of diabetic nephropathy. It also has 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 members of the 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 a combination pill with other antihypertensive agents including IRBE.[7-9]

Combined cardiovascular therapy is beneficial, since most of these therapies exploit complementary mechanisms of differently 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 the present work, a liquid chromatographic method for the simultaneous determination of IRBE and HCT was developed using reverse phase high performance liquid chromatography (RP-HPLC). Experimental conditions such as different buffer solutions, various pH values, temperature, additives, and 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.

MATERIALS AND METHODS

Chemicals and instruments

IRBE and HCT in their dosage forms Karvezide® and Co-Irda® were obtained from pharmaceutical companies. All reagents were of analytical grade and were prepared using 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, and phosphoric acid were used. All other reagents were of analytical grade.

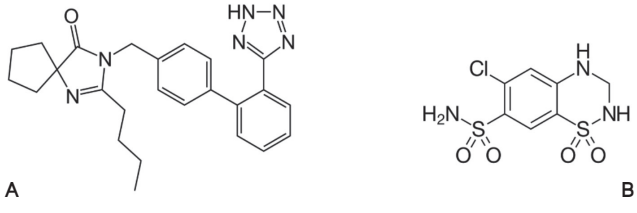

Figure 1. Chemical structures of A) irbesartan and B) hydrochlorothiazide

The 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, with an X Terra RP® (250x5 mm I.D.: 3 µm) column at a flow rate of 0.8 mL min^{-1} and an injection value of 10 µL. The separation was carried out at 30 °C and the diode array detector was adjusted to 226 nm. Before using the mobile phase, the pH was adjusted with 5 M NaOH, and the mobile phase filtered through 0.45-µm polytetrafluoroethylene membranes using a vacuum pump and degassed. For the pH measurements, a pH meter Model 538 (WTW, Weilheim, Germany) was used with a combined electrode with an accuracy of ±0.05 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 of methanol by ultrasonication for 10 min in an 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 contents of IRBE and HCT were calculated from the related calibration curves.

Statistical Analysis

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 the United States Pharmacopeia (USP) criteria.[18-20] Validation of the proposed method was performed according to International Council on Harmonisation (ICH) Guidelines and USP criteria in terms of precision, accuracy, linear range, limit of detection (LOD), and quantification values and reported.

Degradation studies

It is recommended in the ICH Guidelines to perform degradation studies for the developed assays to show that 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 harsh conditions like heating in an oven (75 °C, 3 h and 24 h), treating the samples with acidic

(0.5 N HCl and 1 N HCl) or alkaline (0.5 N NaOH and 1 N NaOH) solutions or oxidants (3% and 30% H_2O_2), and exposing to ultraviolet (UV) (254 nm, 3 h and 24 h) were applied.

RESULTS AND DISCUSSION

System suitability test parameters

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

Method validation

Linearity

The 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). Repeated injections were performed at each concentration level. The linear range was in good agreement and wide linear range values were obtained. For HCT the linear range obtained was between 0.25 and 25 µg mL^{-1} and for IRBE it was between 0.1 and 25 µg mL^{-1} (Figure 2).

Limit of detection and limit of quantification

The ICH guidelines propose several approaches for the determination of LOD and limit of quantification (LOQ). In the present work the LOD and LOQ values were calculated as 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] The LOD and LOQ values are also reported in Table 2, where a statistical evaluation of the calibration data is presented. HCT was detected with an LOD value of 0.012 µg mL^{-1} and an LOQ value of 0.036 µg mL^{-1} with the proposed RP-HPLC method. IRBE was detected with an LOD value of 0.008 µg mL^{-1} and an LOQ value of 0.023 µg mL^{-1} with the proposed RP-HPLC method.

Table 1. System suitability tests parameters

	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

HCT: Hydrochlorothiazide, IRBE: Irbesartan, LOD: Limit of detection, LOQ: Limit of quantification, RSD: Relative standard deviation

Precision

The precision of the proposed method was evaluated by 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 on three consecutive days, respectively. The results are expressed by the percentage of relative standard deviation (RSD %) and shown in Table 2, where a statistical evaluation of the calibration data is presented. As seen, there is no significant difference between the values within the within-day and between-day measurements in the mobile phase.

Accuracy

Accuracy is an important parameter 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

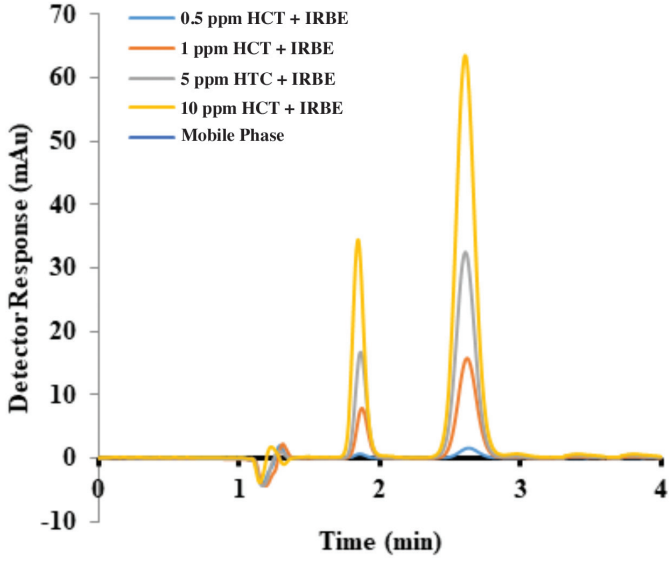

Figure 2. Chromatograms of increasing concentrations of hydrochlorothiazide and irbesartan
HCT: Hydrochlorothiazide, IRBE: Irbesartan

Table 2. Statistical evaluation of the calibration data

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

HCT: Hydrochlorothiazide, IRBE: Irbesartan, Min: Minute

Co-Irda®, which contain 150 mg of IRBE and 12.5 mg of HCT (Table 3). For the accuracy test, recovery of the method was studied using the spiking method. Solutions were prepared by adding 50%, 100%, and 150% level 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 concluded that, using the suggested HPLC method, acceptable recoveries can be obtained with RSD % values lower than 1 and recovery values between 98% and 102%.

Specificity (degradation studies)

Specificity is another essential parameter in pharmaceutical analysis.[19,21-23] Specificity studies were performed by means of degradation studies. Degradation of drugs under mild and drastic stress conditions is shown in Table 5, as degradation %. It can be seen that the hard acidic and alkaline conditions hardly affect these drugs. In UV and heat degradation these drugs can be affected within 3 h (Table 5).

CONCLUSION

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

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 experiments. HCT: Hydrochlorothiazide, IRBE: Irbesartan, RSD: Relative standard deviation

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 experiments, HCT: Hydrochlorothiazide, IRBE: Irbesartan, RSD: Relative standard deviation

Table 5. Results of stress conditions by reverse phase liquid chromatography 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 (3 h at 254 nm)	90.18	90.98
	Oven (3 h at 75 °C)	95.11	72.56
Harsh 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 (24 h at 254 nm)	97.48	78.46
	Oven (24 h at 75 °C)	96.97	87.87

HCT: Hydrochlorothiazide, IRBE: Irbesartan, UV: Ultraviolet

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