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Determination of quality of tablets containing irbesartan and hydrochlorothiazide via newly developed and validated simultaneous HPLC method

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ABSTRACT

Irbesartan and hydrochlorothiazide are common combination drugs used to treat hypertension. The goal of this study was to develop an HPLC method for simultaneous quantification of IRB and HCT and to use this method in tablet quality control tests. The mobile phase in gradient elution mode HPLC method was 30 mM sodium acetate buffer (pH:5.00): water: ACN (40:40:20, *v/v/v %*) at a flow rate of 0.6 mL/min and 230 nm and that employed avanafil as an internal standard. The ICHQ1 (R2) guideline was used to determine its applicability and capacity studies. The tablets were then subjected to weight variation, thickness-width-length tests, hardness tests, content uniformity, and dissolution tests as quality control tests. The mean recovery for hydrochlorothiazide in the accuracy study was 99.76% and 99.10% for irbesartan. The dissolution test results were discovered that 85% of both active substances were released into the dissolution medium within the first 15 minutes.

Keywords: Fixed dosage form, hydrochlorothiazide, irbesartan, tablet quality control tests

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INTRODUCTION

Hypertension is an important public health problem due to its widespread prevalence around the world and the increased risk of death it poses when combined with other diseases [1, 2]. Antihypertensive drugs are used not just to lower blood pressure, but also to eliminate the negative consequences of hypertension [3]. Many different drug classes are used to treat hypertension. Angiotensin-converting enzyme inhibitors (ACE-I), beta-blockers, calcium channel blockers (CCB), thiazide diuretics, and angiotensin receptor blockers (ARB) are the drugs used to treat hypertension [4-6]. If monotherapy with a single antihypertensive drug group fails, most guidelines advise going with a thiazide diuretic and an ARB [5, 7, 8]. In comparison to monotherapy, combined drug treatments use less active substance on the patient. In this way, using combined drugs instead of monotherapy in the treatment of hypertension provides a more effective treatment with fewer side effects [5].

Irbesartan (IRB) (2-butyl-3-[[4-[2-(2H- tetrazol-5-yl)phenyl]phenyl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one) is a non-peptide ARB [9, 10]. Hydrochlorothiazide (HCT) (6-chloro-1,1-dioxo-3,4-dihydro-2H- 1lambda6,2,4-benzothiadiazine-7-sulfonamide)is a thiazide class diuretic [11]. Avanafil (AVA) was used as an internal standard (IS). Figure 1 shows the chemical structures of IRB, HCT and AVA.

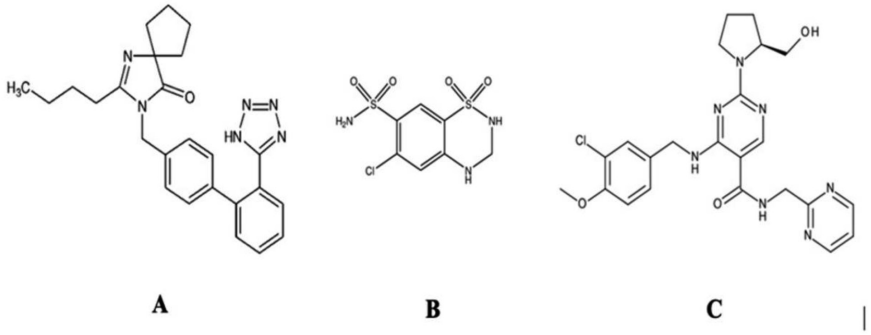

Figure 1. Molecular structure of Irbesartan (A), Hydrochlorothiazide (B), and Avanafil (C)

Various detection methods are still being developed in order to identify, quantify, or purify active substances. It is difficult and time-consuming to determine active substances simultaneously in combined preparations containing more than one active substance [12, 13]. HPLC is one of the most commonly used methods for the analysis of pharmaceutical formulations and body fluids due to its advantages, ease of application, and availability of low-cost instruments. Creating an effective method ensures that laboratory resources are optimized

while meeting the routine goals that must be met at each stage of drug development [13].

Our study aims to develop a validated High-performance liquid chromatography (HPLC) method for the simultaneous quantification of IRB and HCT, as well as to perform Quality Control (QC) tests on all combined IRB/HCT tablets on the market.

METHODOLOGY

Chemicals

All of the chemicals and solvents used were analytical reagent grade. Acetonitrile (ACN) and distilled water were purchased from Merck KGaA (Darmstadt, Germany). Milli-Q water is purified using Millipore SAS's Millipore Milli-Q Synthesis A10 system (Molsheim, France). Sodium acetate, acetic acid and sodium hydroxide was purchased from Sigma-Aldrich Chemie GmbH (Darmstadh Germany). Standards for IRB, HCT and AVA were obtained from Molekula GmbH (Munchen, Germany). IRB/HCT (300/25 mg) fixed-dose combination drug product was supplied from local pharmacies.

Preparation of the calibration standards

The standard stock solution of IRB (930 μg mL^{-1}) and HCT (540 μg mL^{-1}) were prepared in ACN. The working standard solutions (9.3, 27.9, 74.4, 148.8, 186, 223.2, 279, 334.8, 372 and 409.2 μg mL^{-1} for IRB) (5.9, 17.7, 47.2, 94.4, 118, 141.6, 177, 212.4, 236, 259.6 μg mL^{-1} for HCT) were prepared by diluting the stock solution in the ACN. The stock solution was kept at +4°C where it is stable for at least one week. Standard solutions were daily prepared by diluting the stock with ACN.

Preparation of the QC samples

Ten tablets were weighed and ground to determine the average weight. Amount of powder equivalent to average weight was transferred to a 250 mL volumetric flask, 200 mL of diluent (ACN %100) was added, and sonicated for 15 minutes. The volume was diluted to produce a solution containing 1200 μg mL^{-1} IRB and 100 μg mL^{-1} HCT. Before injecting the solution into the HPLC system, it was filtered through a 0.20 μm PTFE membrane filter and diluted with diluent to 300 μg.mL^{-1} IRB and 25 μg mL^{-1} HCT. The QC samples were kept frozen (at -18 °C) until used, and calibration samples were prepared fresh for each batch.

Preparation of the mobile phase

For use in the mobile phase, a 30 mM sodium acetate buffer solution (pH 5.00) was prepared. Acetic acid and sodium hydroxide were used to adjust the pH to 5.00. For 20 minutes, it was sonicated. It was then filtered with 0.45 µm non-sterile cellulose acetate membrane filter paper using a vacuum filtration equipment and degased before use.

Chromatic equipment and conditions

HPLC analysis was performed on a chromatographic system equipped with Nexera-i LC 2040C 3D device from Shimadzu (Japan). The chromatographic data were collected, integrated, and analyzed using a LabSolutions Software data system. PDA detection was performed with the wavelength set to 230 nm and the real-time spectra were recorded at 640 msec data sampling. C18 core-shell column (SUPELCO® Ascentis Express, 100 × 4.6 mm, 2.7 µm i.d.) was used for separation. AVA was used as an IS to quantify IRB and HCT since the AVA peak did not interfere HCT and IRB and the retention time was longer than the targeted compounds.

In gradient elution mode, the mobile phase was 30 mM sodium acetate buffer (pH:5.0) : water : ACN (40:40:20, *v/v/v %*) at a flow rate of 0.6 mL.min^{-1} and injection volume was 1 µL. The column ovent temperature was set 30 °C. Table 1 shows the gradient elution conditions.

Table 1. Gradient elution conditions

Time (min)	ACN (%)	Buffer (%)
0.00-3.50	20.0 → 60.0	40
3.50-4.00	60.0	40
4.00-4.50	60.0 → 20.0	40
4.50-8.00	20.0	40
8.00	Stop	

Method validation

To determine the applicability and capacity of the analytical method used, linearity, accuracy, precision, selectivity and specificity, sensitivity, and robustness tests were performed as part of the validation studies. Validation studies were conducted following ICH guidelines and published literature [14, 15].

Specificity

Method trials were carried out to ensure the specificity of the method and to avoid interference between the compound to be analyzed and other peaks in the medium.

Linearity and sensitivity

For the linearity study, triplicate measurements at 10 different concentrations (9.3, 27.9, 74.4, 148.8, 186, 223.2, 279, 334.8, 372 and 409.2 μg mL^{-1} for IRB) (5.9, 17.7, 47.2, 94.4, 118, 141.6, 177, 212.4, 236, 259.6 μg mL^{-1} for HCT) were plotted against the ratios of the peak areas of IRB and HCT to IS, and the calibration curves were obtained. The calibration curve's line equation and the regression coefficient (r^2) were calculated.

Limit of detection (LOD) and limit of quantification (LOQ) values were calculated to demonstrate the analytical sensitivity of the method. The ICH guidelines LOD and LOQ formulas were used for the calculation [14].

Accuracy and precision

The accuracy value expresses the relationship between the measured value and the values contained in the analyte. The fact that the measurements of the series obtained after sampling the same sample multiple times under the same conditions demonstrates the precision of the method. Intra-day and inter-day recovery studies were carried out to demonstrate the accuracy and precision of the method.

The assay accuracy of the method was determined for intra-day variations using ten times analysis and inter-day variations using twenty times analysis of samples containing 148.8, 186, 232.2 μg mL^{-1} IRB and 94.4, 118, 141.6 μg mL^{-1} HCT. Three different concentrations of standard solutions (within the linear range) were analyzed on three consecutive days (inter-day precision) and ten times within the same day (intra-day precision). The obtained values for relative standard deviation (RSD) and Bias of intra- and inter-day studies.

Robustness

The analytical method used expresses the robustness of the rate of being affected by small changes in the method's parameters. At the same time, it demonstrates how reliable the method is during application. The robustness of the analytical method was determined by making changes to the flow rate, the percentage of organic solvent in the mobile phase, the buffer capacity used in the mobile phase, the column temperature, and the wavelength.

Quality Control Tests

In the Turkish pharmaceutical market, QC tests were performed on fixed dose tablets of 300/25 mg (IRB/HCT). There were tablets from three different companies. These tablets were assigned codes A, B, and C at randomly. Weight variation, thickness-width-length test, hardness tests, content uniformity, and dissolution tests were used to demonstrate the quality control (QC) of the tablets. All tablet QC tests were performed in accordance with the guidelines and literature [16-18].

RESULTS and DISCUSSION

Method optimization

HPLC is one of the most widely used methods for drug analysis in pharmaceutical preparations and physiological fluids due to advantages such as simplicity of use and low cost [13, 19]. HCT, one of the pharmaceutical active substances quantified, is much more polar than IRB, another pharmaceutical active substance [12, 20, 21]. In our experiments, we noticed that HCT elutes rapidly. During the simultaneous determination of active substances, it was noticed that IRB eluted too late due to the polarity difference among IRB and HCT, while trying to remove HCT from dead time and obtain a capacity factor greater than one.

The outcomes of the preliminary study shows that gradient elution should be used. The gradient elution conditions were used after all optimization studies. Elution was finalized in less than 5 minutes using this method. The method required the use of an IS. IS has been tried with a variety of molecules. However, AVA was used as IS because it did not interact with IRB and HCT and eluted relatively late from HCT and IBR to an acceptable degree. Under optimized conditions, the required specificity for the HPLC methods HCT, IRB, and AVA was successfully accomplished. Figure 2 shows the chromatogram that was used to separate the obtained peaks.

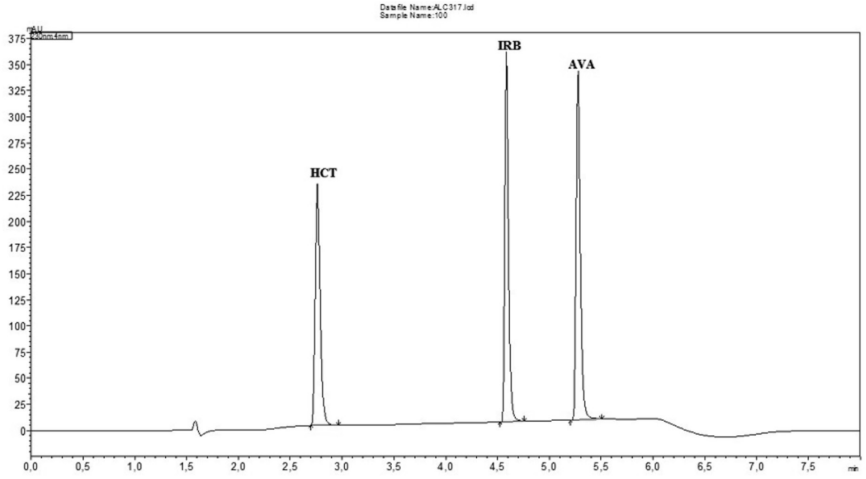

Figure 2. Chromatogram of the peaks of IRB, HCT, and AVA

Peaks from three different substances could be eluted separately, for a total analysis time of eight minutes. The wavelength used for analysis was 230 nm, and all three active substances produced strong signals at this wavelength. The injection volume was selected to be 1 µL. Under optimized experimental conditions and 1 µL injection volume, the symmetry of the peaks and theoretical layer number were calculated as 11817 for HCT and 63038 for IRB, and these results were within acceptable limits (N>2000). The analytical method's system suitability data were calculated, and the results are shown in Table 2. Our analytical method is successful, as demonstrated by the process parameter and chromatograms.

Table 2. The system-suitability data for HCT (118.0 µg mL-1) and IRB (186.0 µg mL-1)

Parameter	Obtained Value		Acceptance Criteria
	HCT	IRB	
Retention Time (min)	2.76	4.59	-
Relative Retention Time (min)	0.52	0.87	-
Standard Deviation (%) of Relative Retention Time	0.01	0.02	RSD[b] ≤1%
Precision for Relative Area	0.24	0.35	RSD[b] ≤ 1%
Injection Precision for Retention Time (min)	0.04	0.05	RSD[b] ≤ 1%
Theoretical Number of Plates (N)	11817	63038	N > 2000
Resolution (Rs)	13.91	20.87	>2
Tailing Factor (T)	1.29	1.39	≤2
USP Widtha	0.10	0.07	≤1
HETPc	12.69	2.38	-

[a] Calculated according to USP.

[b] RSD: Relative Standard Deviation (%).

[c] HETP: Height Equivalent to One Theoretical Plate.

Method validation

The calibration curve's was determined with peak normalization method and regression coefficients for both analyte were calculated 0.99 as a good linearity. Also; for n=10 and n=30, intraday and interday precision studies were carried out separately. The study results in Table 3 show that the analytical method provides precise results.

Table 3. Statistical data for the linearity, sensitivity, and precision studies of HCT and IRB

Parameter	HCT	IRB
Linearity Range (μg mL^{-1})	5.90 - 259.6	9.30 – 409.2
Slope (intraday, n=10)	12.766	0.9687
Intercept (intraday, n=10)	0.019	0.003
Regression Coefficient (intraday, n=10)	0.9980	0.9996
Standard Error of Slope (intraday, n=10)	0.020	0.007
Standard Error of Intercept (intraday, n=10)	0.016	0.009
Slope (interday, n=30)	1.277	0.974
Intercept (interday, n=30)	0.022	-0.002
Regression Coefficient (interday, n=30)	0.9979	0.9986
Standard Error of Slope (interday, n=30)	0.011	0.007
Standard Error of Intercept (interday, n=30)	0.009	0.009
LOD (ng/mL)	0.094	0.053
LOQ (ng/mL)	0.284	0.160
ANOVA	F (2.27)=0.0004 P=0.9996 (P>0.05)	F (2.27)=2.76×10^{-5} P=0.9999 (P>0.05)

The LOD and LOQ calculated using the ICH guideline equation were 0.094 μg mL^{-1} and 0.284 μg mL^{-1} for HCT, and 0.053 μg mL^{-1} and 0.160 μg mL^{-1} for IRB, respectively [14]. All calculated values were found to be significantly below the lowest concentration in the range, demonstrating that the analytical method developed sensitive results for both HCT and IRB.

Table 4 shows the outcomes of the analytical method's accuracy studies. The relative standard deviation (RSD) was found to be less than 2% in all of the results obtained. The mean recovery in the accuracy study was 99.76% for HCT and 99.10% for IRB. Because all of the results are within the range of 100%±2, it is assumed that the technique provides accurate results.

Table 4. Statistical evaluation of accuracy studies

Compound	Added Concentration (µg mL^{-1})	Measured Concentration (µg mL^{-1})	Recovery (%)	Standard Deviation	Relative Standard Deviation (%)	Recovery Error (%)	Mean Recovery (%)
HCT	94.4	95.3	100.9	1.15	0.41	0.91	99.76
	118.0	116.3	98.6	1.51	0.57	-1.45	
	141.6	141.8	100.2	0.79	0.42	-0.19	
IRB	148.8	146.3	98.3	0.47	0.88	-1.77	99.10
	186.0	185.9	99.9	1.28	0.33	-0.04	
	223.2	221.0	99.0	0.58	0.39	-0.10	

Considering the method robustness parameters, it was calculated that the most change was in the HCT peak area according to the detection wavelength. In the organic phase and flow rate changes, the retention time of AVA changed more. In the resolution change, both the analytes and the internal standard were less affected. The robustness date calculated for each compounds are as given in the Table 5.

Table 5. Robustness data *(n=3)*

		Retention time (min)		Peak area		Resolution	
		Observed value	Difference (%)	Observed value	Difference (%)	Observed value	Difference (%)
				HCT			
Column temperature °C	27	2.9	3.3	755548	-1.0	14.0	2.2
	33	2.7	-3.3	749505	-1.8	13.1	-4.3
Flow rate (mL/min)	0.54	3.1	10.7	842267	10.3	14.3	4.5
	0.66	2.5	-8.6	690832.7	-9.4	13.0	-5.2
Organic phase (%)	18	3.0	7.8	750093	-1.7	16.5	20.4
	22	2.6	2.6	758175	-0.7	11.6	-15.4
Buffer (%)	36	2.8	0.2	754176	-1.1	13.7	-1.2
	44	2.8	0.3	755485	-1.0	14.1	2.7
Detector wavelength (nm)	226	2.8	0.1	1115274	46.1	13.7	0.1
	234	2.8	0.1	306169	-59.8	14.2	3.8

	IRB						
Column temperature °C	27	4.6	0.1	866188	-0.8	19.9	-4.7
	33	4.6	0.4	860137	-1.5	21.8	4.5
Flow rate (mL/min)	0.54	4.9	6.8	969770	11.1	20.2	-3.3
	0.66	4.3	-5.2	793683	-9.1	22.0	5.6
Organic phase (%)	18	4.9	8.8	869550	-0.4	23.3	11.6
	22	4.3	-6.9	870331	-0.3	18.7	-10.3
Buffer (%)	36	4.6	0.2	855813	-2.0	21.0	0.6
	44	4.6	0.3	865022	-0.9	20.8	-0.5
Detector wavelength (nm)	226	4.6	-0.6	891133	2.1	20.9	0.0
	234 n	4.6	0.2	815615	-6.6	21.0	0.5
	AVA						
Column temperature °C	27	5.3	-0.3	947496	-1.3	8.5	-5.3
	33	5.3	0.4	944593	-1.6	9.2	2.9
Flow rate (mL/min)	0.54	5.6	6.3	1058900	10.3	8.7	-2.3
	0.66	5.0	-5.2	868366	-9.5	8.8	-2.0
Organic phase (%)	18	4.9	-6.9	952325	-0.8	9.0	1.0
	22	5.7	8.4	942416	-1.8	8.4	-5.7
Buffer (%)	36	5.3	0.1	951492	-0.8	8.7	-2.7
	44	5.3	0.1	948365	-1.2	8.7	-2.7
Detector wavelength (nm)	226 nm	5.3	0.0	903618	-5.8	9.0	0.0
	234 nm	5.3	0.0	1017867	6.1	9.0	0.0

Quality Control Tests

Table 6 and Table 7 show the calculated results of the tablet quality control tests. and obtained chromatogram was given Figure 3.

Table 6. Weight variation results (n=20)

Parameters	Drug A	Drug B	Drug C
Acceptable Range (±%5) (mg)	596.28 - 659.05	590.75 - 652.93	579.20 - 640.16
Minimum Tablet Weight (mg)	610.63	614.48	598.78
Maximum Tablet Weight (mg)	643.97	632.16	616.82
Average Tablet Weight (mg)	627.67	621.84	609.68
Standard Deviation	8.95	5.07	4.35

Table 7. Content uniformity results (Mean ± SD) (mg)

	HCT	IRB
Drug A	22.70 ± 0.17	293.15 ± 1.03
Drug B	22.66 ± 0.07	289.79 ± 0.30
Drug C	21.83 ± 0.08	286.66 ± 0.75

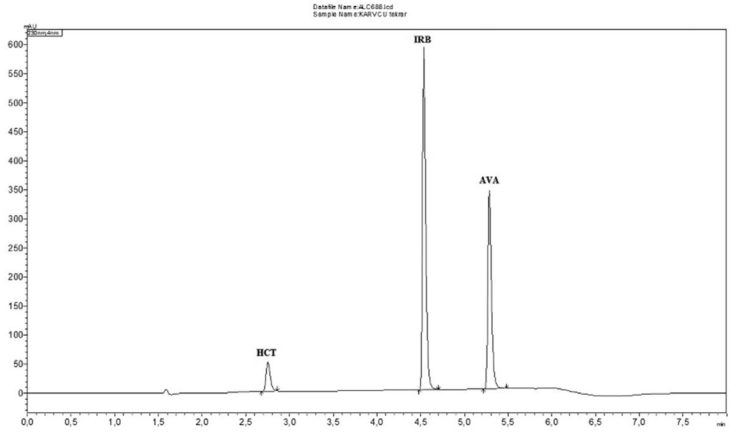

Figure 3. The obtained chromatogram of quality control test

The tablet weight variation was determined to be 610.63 – 643.97 mg for Drug A, 614.48 – 629.12 mg for Drug B, and 598.78 – 616.82 mg for Drug C. For tablets weighing more than 250 mg, the acceptable weight distribution is ±5%. A maximum of two tablets could be within the ±5% range on average among the weight measured values on 20 tablets. No tablet, however, should outweigh the ±10% range [14]. When the data was analyzed, all of the tablets from three different brands complied with the specifications.

The content uniformity test was carried out on six tablets. Each tablet was individually weighed and disintegrated in a volumetric flask with 250 mL of ACN in a sonicator for 20 minutes. Content uniformity test results shown in Table 7, RSD values in all companies were calculated to be less than 2%.

Table 8 shows the thickness, width, and length measurements. The obtained data show that the tablets are self-consistent.

Table 8. Thickness-Width-Length test and Hardness test results (n=10)

	Drug A	Drug B	Drug C
Thickness (Mean ± SD) (mm)	5.58 ± 0.09	5.95 ± 0.01	5.73 ± 0.03
Width (Mean ± SD) (mm)	9.39 ± 0.09	9.31 ± 0.02	9.32 ± 0.04
Length (Mean ± SD) (mm)	17.49 ± 0.13	17.54 ± 0.08	17.48 ± 0.12
Hardness (Mean ± SD) (Newton)	143.12 ± 33.15	118.57 ± 8.03	129.60 ± 12.21

In accordance with the pharmacopea dissolution study, 1000 mL of 0.1 N HCl was used for 45 minutes using apparatus 2 at a rotation speed of 50 rpm. The cumulative drug concentration (%) was calculated using samples taken at 5, 10, 15, 20, 30, and 45 minutes. Figure 4 and Figure 5 indicate the dissolution profiles.

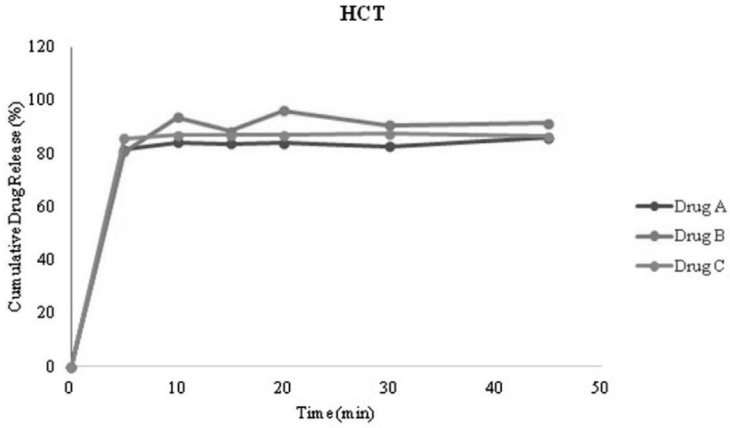

Figure 4. Dissolution profiles of HCT (The error bars indicate SD.) (n=6)

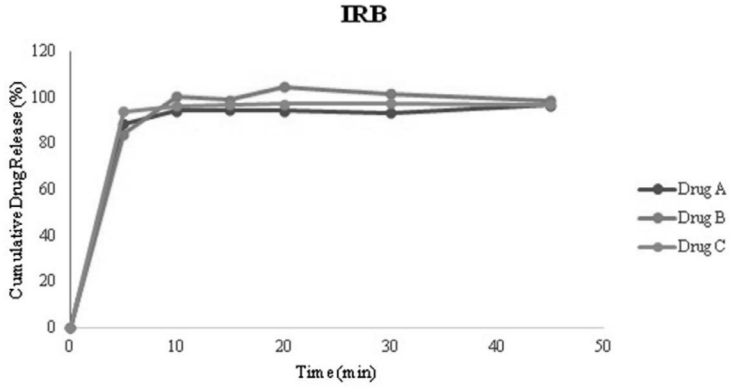

Figure 5. Dissolution profiles of IRB (The error bars indicate SD.) (n=6)

We worked with film-coated tablets that immediately released (IR). In the first 15 minutes, IR tablets should release 85% of the drug content [22]. When we evaluate the dissolution profiles, we show that 85% of the IRB is released within the first 10 minutes in each of Drug A, Drug B, and Drug C. HCT, on the other hand, reached 85% in Drug A before the 5th minute and within the first 10 minutes in Drugs B and C.

It is critical to meet the validation conditions so that the analytical methods planned for quantification can produce accurate, precise, and sensitive results. We developed a gradient elution HPLC method with AVA as an internal standard in this study. In addition, we have fully validated this method by the ICHQ2(R1) guideline.

This analytical method that we developed is a simple and low-cost method for simultaneously quantifying IRB and HCT.

We discovered that all 300/25 mg, IRB/HCT-containing tablets in the Turkish pharmaceutical market met all of the requirements when we examined the data from the weight variation, thickness-width-length test, hardness test, content uniformity, and dissolution tests, which we performed later.

STATEMENT OF ETHICS

Not applicable as no human or animal subjects were involved in the study.

CONFLICT OF INTEREST STATEMENT

The authors declare there is no conflict of interest associated with this study.

AUTHOR CONTRIBUTIONS

Concept: MSK (Mustafa Sinan Kaynak), SÖ (Saniye Özcan); Design: MSK, SÖ; Supervision: MSK, SÖ, MÇ (Mustafa Çelebier); Materials: BK (Berna Kaval), SÖ; Data collection and/or processing: BK, SÖ; Analysis and/or interpretation: BK, SÖ, MSK; Literature search: BK; Writing: BK, SÖ, MÇ; Critical reviews: SÖ, MSK, MÇ.

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Not Applicable

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