Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Tablets by CE-DAD

İrbesartan ve Hidroklorotiyazidin CE-DAD ile Tabletlerden Eşzamanlı Miktar Tayin

Research Article

Arin Gül Dal*, Sema Koyutürk
Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskişehir, Turkey.

ABSTRACT

A capillary electrophoretic method was developed and validated for the simultaneous determination of irbesartan and hydrochlorothiazide in tablets. The run buffer was used 10 mM borate buffer containing 10% methanol (pH 9.0). Compounds were separated less than 6 minutes. The limit of quantification (LOQ) values were 3.370×10^{-7} M (0.144 µg/mL) and 3.140×10^{-7} M (0.094 µg/mL) for irbesartan and hydrochlorothiazide, respectively. The method was applied successfully to both irbesartan and irbesartan/hydrochlorothiazide combined tablets with good recoveries almost 100%.

Key Words
Irbesartan, hydrochlorothiazide, capillary electrophoresis, validation.

ÖZET

İrbesartan ve hidroklorotiazidin tabletlerde eş zamanlı analizi için bir elektroforetik yöntem geliştirilmiştir. %10 metanol içeren 10 mM borat (pH 9.0) çalışma tamponu olarak kullanılmıştır. Bileşikler 6 dakikadan daha düşük sürede ayrılmıştır. Tayin sınırı (LOQ) değerleri irbesartan için 3.370×10^{-7} M (0.144 µg/mL) ve hidroklorotiazid için 3.140×10^{-7} M (0.094 µg/mL)'dir. Yöntem, irbesartan ve irbesartan/hidroklorotiazidi birlikte içeren tabletlere yaklaşık %100 gibi bir geri kazanımla başarılı bir şekilde uygulanmıştır.

Anahtar Kelimeler
İrbesartan, hidroklorotiazid, kapiler elektroforez, validasyon.

Article History: Received: Apr 15, 2015; Revised: June 19, 2015; Accepted: Jul 20, 2015; Available Online: Oct 31, 2015.

DOI: 10.15671/HJBC.20154314235

Correspondence to: A.G. Dal, Anadolu University, Faculty of Pharmacy, Department of Analytical Chemistry, Eskişehir, Turkey.
Tel: +90 222 335 3380 Fax: +90 222 335 0750 E-Mail: agdal@anadolu.edu.tr

INTRODUCTION

Irbesartan (IRB), 2-butyl-3-({4-[2-(2H-1,2,3,4-tetrazol-5-yl)phenyl]phenyl}methyl)-1,3-diazaspiro[4.4]non-1-en-4-one, is an angiotensin II receptor antagonist used for the treatment of hypertension [1]. Hydrochlorothiazide (HCT), 6-chloro-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide-1,1-dioxide, is a thiazide diuretic and used alone or in combination with antihypertensive drugs in hypertension treatment [2].

Several analytical methods have been reported for the determination of IRB and HCT including spectrophotometric [3-7] and chromatographic [8-18] methods in pharmaceutical formulations and/or biological fluids. Although current literature are primarily based on spectrophotometric and chromatographic methods, capillary electrophoresis is a promising technique for drug analysis. But only one capillary electrophoretic method [19] has been reported which suffers from long analysis time and some validation parameters. So there is definitely need for an analysis with better run time and validation.

Capillary electrophoresis has the advantages of providing fast and efficient separations with small sample volumes and low reagent consumption. The aim of the study is to develop and validate a simple, sensitive and selective capillary electrophoretic method for the determination of IRB and HCT simultaneously.

MATERIALS AND METHOD

Chemicals

USP certificated IRB and HCT were purchased from Referans Kimya (Turkey) (Figure 1). Candesartan (CAN) was kindly supplied by Astra Zeneca (Sweden) and used as internal standard (IS). All other chemicals were analytical grade and of Merck (Germany). Ultrapure water was obtained by a Milli-Q system of Millipore (U.S.A.).

Instruments

Electrophoretic experiments were performed by an Agilent 7100 model capillary electrophoresis (Agilent Technologies, U.S.A.) with a photo diode array detector. The compounds were monitored at 230 nm. Separations were achieved by a capillary (Agilent Technologies, U.S.A.) of 40 cm effective length (48.5 cm total) and 50 µm i.d. The analytical balance and pH-meter were from Mettler-Toledo (Switzerland). The solutions were sonicated by a L30 model sonicator (Ultrasonics, Germany), vortexed by a VNI-96B model vortex (Jeio Tech, Korea) and centrifuged by a EBA20 model (Hettich, Germany) centrifuge. All solutions were filtered by a 0.45 µm filter (Carl Roth, Germany) before injection.

Preparation of Solutions

The stock solutions of IRB, HCT and CAN were prepared in methanol and stored in -20°C. The dilutions were made by a solution of 1/10 run buffer to the appropriate volume. The borate buffer was prepared by weighing the appropriate mass of disodium tetraborate and it was dissolved in water. The run buffer was 10 mM borate buffer containing 10% methanol and pH was adjusted to 9.00 with 0.1 M HCl. The run buffer was diluted 1/10 with water and this solution was used to dilute the standard solutions.

Capillary was washed with 1 M NaOH solution when first used. Every working day capillary was flushed with 0.1 M NaOH, water and run buffer for 10 minutes, respectively. Between each run capillary was rinsed with the same solutions for 2 minutes.

For the optimization studies a solution with final concentrations of 3.64×10^{-6} M IRB, 3.39×10^{-6} M HCT and 2.52×10^{-6} M IS was employed.

The tablets (Arbesta®, containing 150 mg IRB and Arbesta Plus®, containing 300 mg IRB/12.5 mg HCT) were purchased from local pharmacy. For each tablet, 10 tablets were weighed to calculate the average tablet weight. They were grounded and powdered. An average tablet amount was weighed and dissolved in methanol. The tablet solution was then vortexed, sonicated and centrifuged for 10 minutes at 5000 rpm. The supernatant was diluted with 1/10 run buffer solution to the desired concentration. The solutions were filtered before injection. Three levels of concentrations were prepared for each drug and each solution was analyzed as six replicates.

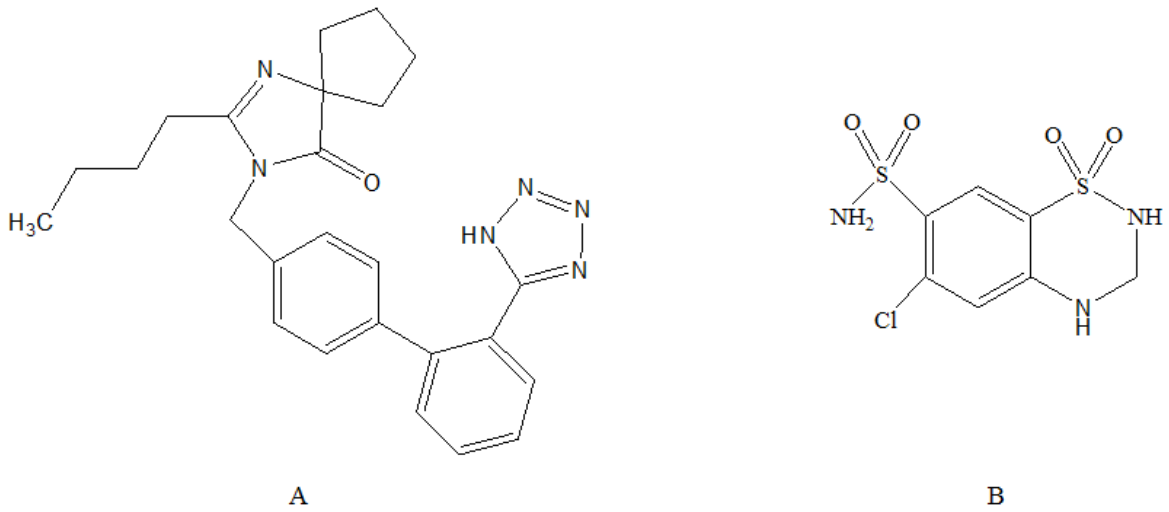

Figure 1. Chemical structures of A) Irbesartan and B) Hydrochlorothiazide.

RESULTS AND DISCUSSION

Optimization of the method

To find out the optimum conditions for electrophoretic separation of drugs, pH of the run buffer, buffer concentration, organic solvent ratio of run buffer, applied potential and injection time and pressure parameters were investigated.

For the electrophoretic separations compounds should be ionized. Considering the pKa values of the drugs [17], the pH of the run buffer should be slightly basic to get the sufficient ionization. The buffering capacity of borate buffer was suitable for this pH range and therefore borate buffer was used as the run buffer.

The pH of the run buffer was investigated in the range of pH 8.50-9.50. Well-shaped peaks with reasonable peak areas and peak resolution appeared at pH 9.0.

Buffer concentration was tested in the concentration range of 10-25 mM borate buffer. Higher buffer concentrations cause high current due to Joule heating. It is best to select the minimum buffer concentration with less generated current and efficient separation. Total run time increased with the increase of run buffer concentration. 15 mM borate concentration gave the best separation of the drugs.

The addition of an organic modifier can improve separation. Because of the drugs were dissolved in methanol, this organic modifier was added to run buffer in the range of 5-15%. Best results were obtained with the addition of 10% methanol.

The applied potential was examined in the range of 20-27.5 kV. Although short analysis times were obtained at 27.5 kV potential, the resolution of the peaks decreased at this potential. Since the maximum potential is preferred in capillary electrophoresis, 25 kV was applied for the optimum results.

The injection pressure and time was 50 mbar for 5 seconds. The longer times and/or the higher pressures caused zone broadening resulting current generation and poor resolution.

The optimum separation was achieved with 10 mM borate buffer containing 10% methanol (pH 9.0) as the run buffer under the applied potential of 25 kV and the standards were injected at 50 mbar pressure for 5 seconds.

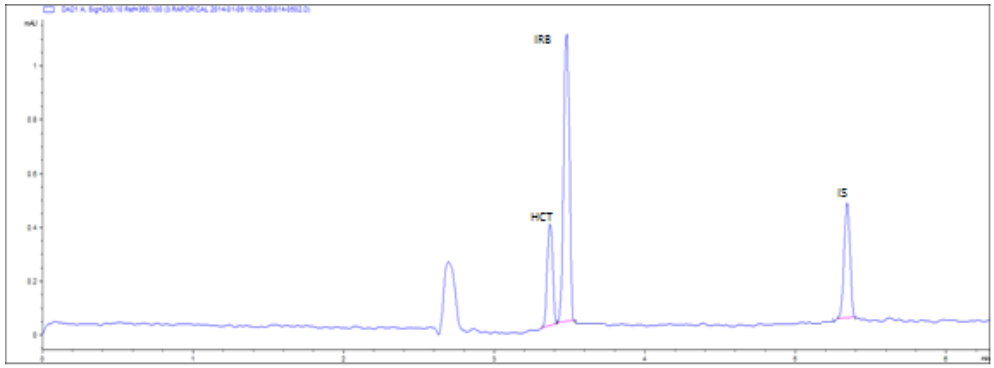

Figure 2. The electropherogram of IRB and HCT under optimum conditions.

Table 1. Precision of the method.

		Intraday		Interdays	
		Day I (n=6)	Day II (n=6)	Day III (n=6)	Wholeday (n=18)
HCT	Mean	11.889	11.718	11.634	11.747
	SD	0.006	0.015	0.016	0.016
	RSD%	0.514	1.293	1.404	1.413
IRB	Mean	33.698	33.592	34.130	33.806
	SD	0.039	0.055	0.031	0.047
	RSD%	1.170	1.639	0.916	1.390

SD: Standard Deviation, RSD: Relative Standard Deviation

Under these conditions the migration times were3.47±0.04,3.37±0.04and5.26±0.03minutes for IRB, HCT and IS (Rs calculated for IRB and HCT was 2.34), respectively. An electropherogram of optimum conditions is given in Figure 2.

Validation of the method

The method was validated in accordance with International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) [20] requirements. The final concentration of IS was 2.520×10^{-6} M in all validation experiments.

Linearity was investigated in the range of 2.512×10^{-7}-1.192×10^{-4} M for HCT and 2.696×10^{-7}-1.280×10^{-4} M for IRB with 11 standard solutions. Good correlations with high coefficient of variation were obtained for both IRB and HCT. Calibration plots were selected in this range. The coefficient of variation of inter-day experiments for calibration experiments were 0.9993 and 0.9988 for IRB and HCT, respectively.

For the precision of the method a standard solution containing 4.240×10^{-6} M HCT, 4.552×10^{-6} M IRB and IS was analyzed in three consecutive days with six replicates. The RSD% values were below 2% as seen in Table 1 and showing that the method is precise.

Accuracy studies were conducted with three different concentrations for both IRB and HCT. Each solution was injected six times a day for three days. The accuracy results of the method are given in Table 2.

The sensitivity of the method was calculated by using the slope of calibration curve and the standard deviation of the response. The LOD values were calculated as 1.021×10^{-7} M (0.044 μg/mL) and 9.515×10^{-8} M (0.028 μg/mL) and LOQ values were calculated as 3.370×10^{-7} M (0.144 μg/mL) and 3.140×10^{-7} M (0.094 μg/mL) for IRB and HCT,

Table 2. Accuracy of the method.

		Added (M)	Found (Mean ± SD)	Recovery %	SE %	RSD %
HCT	Day I (n=6)	5.02×10^{-7}	$5.11\times10^{-7} \pm 7.98\times10^{-9}$	101.96	1.96	1.56
		3.39×10^{-6}	$3.42\times10^{-6} \pm 3.54\times10^{-8}$	101.05	1.05	1.03
		4.28×10^{-5}	$4.27\times10^{-5} \pm 1.76\times10^{-7}$	99.79	-0.21	0.41
	Day II (n=6)	5.02×10^{-7}	$5.09\times10^{-7} \pm 5.36\times10^{-9}$	101.57	1.57	1.05
		3.39×10^{-6}	$3.46\times10^{-6} \pm 1.63\times10^{-8}$	102.12	2.12	0.47
		4.28×10^{-5}	$4.29\times10^{-5} \pm 5.75\times10^{-7}$	100.21	0.21	1.34
	Day III (n=6)	5.02×10^{-7}	$5.18\times10^{-7} \pm 5.14\times10^{-9}$	103.32	3.32	0.99
		3.39×10^{-6}	$3.42\times10^{-6} \pm 4.72\times10^{-8}$	101.07	1.07	1.37
		4.28×10^{-5}	$4.37\times10^{-5} \pm 4.05\times10^{-7}$	102.10	2.10	0.92
	Whole Day (n=18)	5.02×10^{-7}	$5.13\times10^{-7} \pm 7.07\times10^{-9}$	102.28	2.28	1.37
		3.39×10^{-6}	$3.43\times10^{-6} \pm 3.75\times10^{-8}$	101.42	1.42	1.09
		4.28×10^{-5}	$4.31\times10^{-5} \pm 5.93\times10^{-7}$	100.70	0.70	1.37
IRB	Day I (n=6)	5.39×10^{-7}	$5.42\times10^{-7} \pm 5.07\times10^{-9}$	100.56	0.56	0.93
		3.64×10^{-6}	$3.66\times10^{-6} \pm 3.69\times10^{-8}$	100.66	0.66	1.00
		4.55×10^{-5}	$4.58\times10^{-5} \pm 3.13\times10^{-7}$	100.68	0.68	0.68
	Day II (n=6)	5.39×10^{-7}	$5.38\times10^{-7} \pm 5.24\times10^{-9}$	99.79	-0.21	0.97
		3.64×10^{-6}	$3.67\times10^{-6} \pm 2.36\times10^{-8}$	100.82	0.82	0.64
		4.55×10^{-5}	$4.52\times10^{-5} \pm 4.13\times10^{-7}$	99.40	-0.60	0.91
	Day III (n=6)	5.39×10^{-7}	$5.43\times10^{-7} \pm 1.02\times10^{-8}$	100.83	0.83	1.88
		3.64×10^{-6}	$3.67\times10^{-6} \pm 1.81\times10^{-8}$	100.81	0.81	0.49
		4.55×10^{-5}	$4.54\times10^{-5} \pm 3.13\times10^{-7}$	99.71	-0.29	0.68
	Whole Day (n=18)	5.39×10^{-7}	$5.41\times10^{-7} \pm 7.23\times10^{-9}$	100.39	0.39	1.33
		3.64×10^{-6}	$3.67\times10^{-6} \pm 2.59\times10^{-8}$	100.76	0.76	0.70
		4.55×10^{-5}	$4.55\times10^{-5} \pm 4.16\times10^{-7}$	99.93	-0.07	0.91

n: Experiments, SD: Standard Deviation, SE: Standard Error, RSD: Relative Standard Deviation.

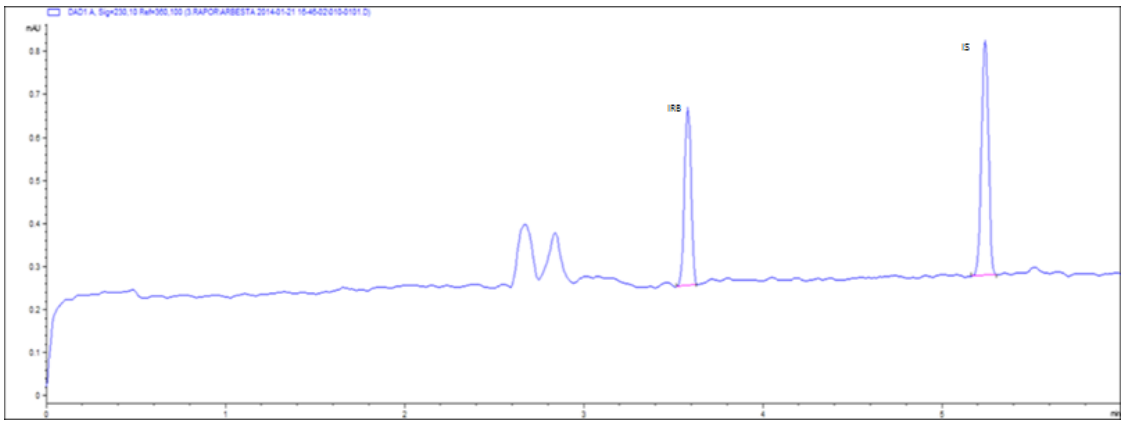

Figure 3. Electropherogram of tablet solution (150 mg IRB).

Table 3. Application of the method to IRB tablets.

	IRB		
Added (M)	3.360×10^{-7}	3.360×10^{-6}	3.360×10^{-5}
Found (M)	3.380×10^{-7}	3.432×10^{-6}	3.380×10^{-5}
SD	5.73×10^{-9}	4.00×10^{-8}	4.51×10^{-7}
RSD %	1.69	1.16	1.33
Recovery %	100.59	102.15	100.61

SD: Standard Deviation, RSD: Relative Standard Deviation

respectively. Stability of the standard solutions was tested by comparing the analysis results of solutions which freshly prepared, stored at room temperature for 24 h and stored at -18°C for one month. The RSD% values were below 2% and both of the drugs were stable under these conditions. The selectivity of the method was proved with checking the signals at different wavelengths during analysis. No interferences with the peaks of interest were observed.

Application of the method

The proposed method was successfully applied to tablets containing 150 mg IRB (Arbesta®) and combined tablets containing 300 mg IRB and 12.5 mg HCT (Arbesta Plus®). The tablet solutions were prepared as described previously and injected to the system. There was no interference originating from the tablet matrixes and the peaks were carrying the characteristics of standard peaks as seen in Figures 3 and 4.

As a result of replicated analyses, peaks of interest were evaluated by peak normalization ratios considering the peak areas and migration times of the drugs and IS. The statistical evaluations of tablet determinations by means of percent recoveries are shown in Tables 3 and 4.

The statistical evaluation of the analyses resulted in good percent recoveries almost 100%. Likewise, the RSD% values were below 2% which is in the acceptable limits [20].

The migration time of the drugs and therefore the total analysis time achieved in this study are better than most of the studies in the literature [9-11,14,19]. Also the LOQ values for the drugs are much better than some of the spectrophotometric [3,4,6] and chromatographic [10,11,14,17] methods published previously. The only capillary electrophoretic method in the literature [19] has reported the migration times as 6.65 and 17.87 minutes for IRB and HCT, respectively. Validation

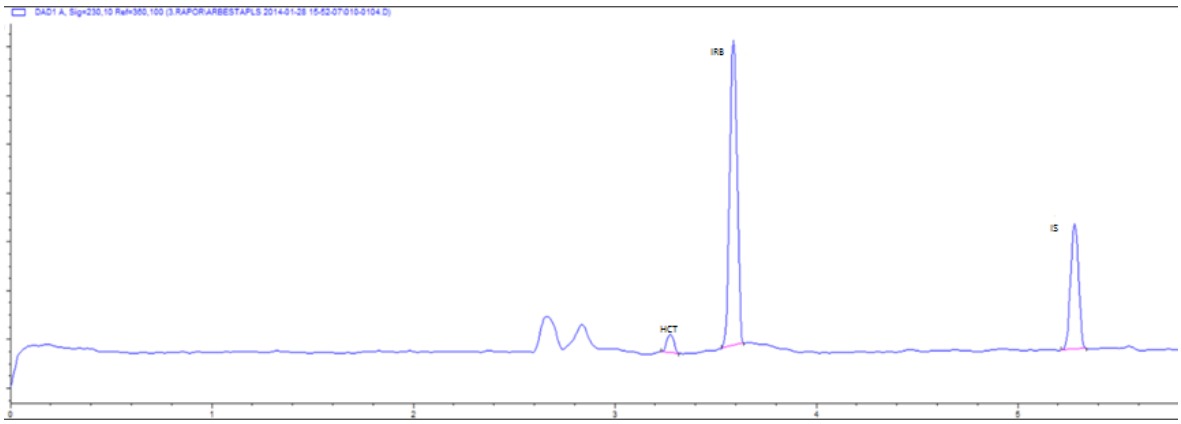

Figure 4. Electropherogram of tablet solution (300 mg IRB /12.5 mg HCT).

Table 4. Application of the method to IRB/HCT tablets.

	HCT			IRB		
Added (M)	4.799×10^{-7}	9.598×10^{-7}	1.440×10^{-6}	8.00×10^{-6}	1.600×10^{-5}	2.400×10^{-5}
Found (M)	4.758×10^{-7}	9.669×10^{-7}	1.435×10^{-6}	7.947×10^{-6}	1.609×10^{-5}	2.467×10^{-5}
SD	5.50×10^{-9}	1.39×10^{-8}	1.96×10^{-8}	1.39×10^{-7}	2.05×10^{-7}	2.31×10^{-7}
RSD %	1.15	1.44	1.36	1.48	1.27	0.93
Recovery %	99.16	100.74	99.68	103.98	100.56	102.8

SD: Standard Deviation, RSD: Relative Standard Deviation

of the method was expressed with only linearity, precision and accuracy parameters. Besides, neither LOQ nor LOD was declared in the study [19], so it was not comparable.

The method developed here is simple, fast, sensitive and selective for the drugs of interest. To the best of our knowledge, this study is the only capillary electrophoretic study with full validation. The method is proposed for the routine simultaneous analysis of IRB and HCT with short analysis time. Besides the method can be applied to biological samples because of low LOQ values for both of the drugs.

ACKNOWLEDGEMENTS

This study was supported by Anadolu University Scientific Research Projects Commission under the grant no: 1207S122.

References

1. M. Burnier, H.R. Brunner, Angiotensin II receptor antagonists, Lancet, 355 (2000), 637-345.
2. A. Lant, Diuretics. Clinical pharmacology and therapeutic use (Part II), Drugs, 29(2) (1985), 162-188.
3. I. Albero, V. Rodenas, S. Garcia, C. Sanchez-Pedreno, Determination of irbesartan in the presence of hydrochlorothiazide by derivative spectrophotometry, J. Pharm. Biomed. Anal., 29 (2002) 299-305.
4. N. Erk, Three new spectrophotometric methods applied to the simultaneous determination of hydrochlorothiazide and irbesartan, Pharmazie, 58 (2003) 543-548.
5. Y.M. Fayez, Simultaneous determination of some anti-hypertensive drugs in their binary mixture by novel spectrophotometric methods, Spectrochim. Acta, Part A: Mol. Biomol. Spectr., 132 (2014) 446–451.
6. J. Joseph-Charles, S. Brault, C. Boyer, M.-H. Langlois, L. Cabrero, J.-P. Dubost, Simultaneous Determination of Irbesartan and Hydrochlorothiazide in Tablets by Derivative Spectrophotometry, Anal. Lett., 36 (2003) 2485 -2495.

7. C. Vetuschi, A. Giannandrea, G. Carlucci, P. Mazzeo, Determination of hydrochlorothiazide and irbesartan in pharmaceuticals by fourth-order UV derivative spectrophotometry, Il Farmaco, 60 (2005) 665–670.
8. A.M. Alanazi, A.S. Abdelhameed, N.Y. Khalil, A.A. Khan, I.A. Darwish, HPLC method with monolithic column for simultaneous determination of irbesartan and hydrochlorothiazide in tablets, Acta Pharm., 64 (2014) 187–198.
9. F.Coudore, L. Harvard, S. Lefeuvre, E.M. Billaud, P. Beaune, G. Bobrie, M. Azizi, P. Prognon, S. Laurent, HPLC–DAD Analysis of Hydrochlorothiazide and Irbesartan in Hypertensive Patients on Fixed-Dose Combination Therapy, Chromatographia, 74 (2011) 559–565.
10. A.A. Elshanawane, L. M. Abdelaziz, M.M. Kamal, H.M. Hafez, Quantitative determination of four angiotensin-II receptor antagonists in presence of hydrochlorothiazide by a gradient technique HPLC in their pharmaceutical preparations, J. Liq. Chromatogr. Related Technol., 37 (2014) 171–186.
11. N. Erk, Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma by liquid chromatography, J. Chromatogr. B, 784 (2003) 195-201.
12. H.M. Hafez, A.A. Elshanawane, L.M. Abdelaziz, M.M. Kamal, Quantitative Determination of three Angiotensin-II-receptor Antagonists in Presence of Hydrochlorothiazide by RP-HPLC in their Tablet Preparations, Iranian J. Pharma. Res., 12 (2013) 635-643.
13. A.S. Khodke, L.V. Potale, M.C. Damle, K.G. Bothara, A validated stability indicating HPTLC method for simultaneous estimation of irbesartan and hydrochlorothiazide, Pharm. Methods, 2(1) (2010) 39-43.
14. S. Koyutürk, N. Ö. Can, Z. Atkoşar, G. Arlı., A novel dilute and shoot HPLC assay method for quantification of irbesartan and hydrochlorothiazide in combination tablets and urine using second generation C18-bonded monolithic silica column with double gradient elution, J. Pharm. Biomed. Anal., 97 (2014) 103-110.
15. X. Qiu, Z. Wang, B. Wang, H. Zhan, X. Pan, R. Xu, Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma by ultra high performance liquid chromatography tandem mass spectrometry and its application to a bioequivalence study, J. Chromatogr. B, 957 (2014) 110-115.
16. L.F. Tutunji, M.F. Tutunji, M.I. Alzoubi, M.H. Khabbas, A.I. Arida, Simultaneous determination of irbesartan and hydrochlorothiazide in human plasma using HPLC coupled with tandem mass spectrometry: Application to bioequivalence studies, J. Pharm. Biomed. Anal., 51 (2010) 985-990.
17. Z. Vujic, N. Mulavdic, M. Smajic, J. Brboric, P. Stankovic, Simultaneous Analysis of Irbesartan and Hydrochlorothiazide:An Improved HPLC Method with the Aid of a Chemometric Protocol, Molecules, 17 (2012) 3461-3474.
18. R. Zhang, X. Chen, Q. Li, W. Liu, W. Yang, K. Bi, L. Sun, Liquid chromatography coupled with mass spectrometry method for the simultaneous quantification of irbesartan and hydrochlorothiazide in human plasma, J. Chin. Pharm. Sci., 20 (2011) 360–367.
19. S. Hillaert, W. Van den Boosche, Simultaneous determination of hydrochlorothiazide and several angiotensin-II-receptor antagonists by capillary electrophoresis, J. Pharm. Biomed. Anal., 31 (2003) 329-339.
20. ICH, Q2(R1), Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology in International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, (2005).