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Research Article

RAPID AND ECONOMICAL QUANTITATIVE DETERMINATION OF SEVERAL ANTIHYPERTENSIVE AGENTS IN PRESENCE OF HYDROCHLOROTHIAZIDE BY ISOCRATIC REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN THEIR PHARMACEUTICAL PREPARATIONS

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ABSTRACT

Objective: Objective of the present investigation is to develop a speedy isocratic reverse phase high-performance liquid chromatography (RP-HPLC) method for the separation and quantitative determination of 5 angiotensin II - receptor antagonists, namely, telmisartan, losartan, valsartan, olmesartan, irbesartan, and atenolol along with thiazide diuretics mostly hydrochlorothiazide (HCTZ).

Methods: RP-HPLC method was evolved using Welchrom C_{18} column (4.6 × 250 mm, 5 µm) as a stationary phase with the mobile phase comprising a variety of phosphate buffer with pH-3.3 and acetonitrile in the proportion of 50:50 v/v. The mobile phase was pumped at a current rate of 1 mL/minute. The detection wavelength was carried out at 230 nm.

Results: The total run time was 6 minutes and the elution window of only 3 minutes. The peaks were eluted with decorous resolution. The calibration curves were linear (r^2=0.9998) in all cases. The percentage relative standard deviation (RSD%) was <2% and average recovery was above 99.95%. The method was validated specificity, precision, and accuracy. High recovery values and low RSD% prove that this method is very accurate and reproducible. The developed method was applied to the estimation of the above-said drugs in binary combinations from different manufacturers which were a good agreement with label claim.

Conclusion: The important advantage of developed method was that the five individual drugs can be determined on a single chromatographic system without alteration in detection wavelength and mobile phase composition. This novel method was statistically validated as per ICH guidelines. The optimized method proved to be linear, accurate, and robust. Hence, the above said proposed method was found to be a rapid tool for the routine determination of the above-said drugs in alone or combination with HCTZ in quality control analysis without interference of excipients.

Keywords: Telmisartan, Losartan, Valsartan, Olmesartan, Irbesartan, Atenolol, Reversed-phase high-performance liquid chromatography.

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INTRODUCTION

Angiotensin antagonists are the first major innovation in essential hypertension management as a first-line treatment. Antihypertensive agents are a largest drug class and hold a major share of the drug market, as hypertension is a major cause of health problems. Hydrochlorothiazide (HCTZ) acts on both RAS and sympathetic nerve system, thereby creating greater sensitivity to angiotensin receptor blockade (ARBs). This HCTZ is a good selection for use in combination with ARBs. ACE inhibitors are having major problems of cough, when compared to ARBs. All the existing ARBs and atenolol (ATEN) are in fixed-dose combination with HCTZ. According to the available present knowledge, no unique single reversed-phase high-performance liquid chromatography (RP-HPLC) method available for the determination of 5 angiotensin II-receptor antagonists, i.e. ATEN, telmisartan (TELM), losartan (LOSA), valsartan (VALS), olmesartan (OLME), and irbesartan (IRBE) along with thiazide diuretics mostly HCTZ. The present proposed method will help in determination of drugs in a single run, which reduces the analysis time and does not necessarily any separate method for each drug and combined tablet formulation. After a meticulous survey of literature reveals that there were some analytical methods have been reported for the determination of the drugs either individually or in combination with some additional drugs in tablet dosage forms and biological samples based on a wide variety of instruments such as spectrophotometric method [1], capillary electrophoresis [2], HPLC [3-16], LC-mass spectrometry (MS) [17,18], and LC-MS/MS [19-21]. Keeping in view of the complete evaluation the authors aim to develop a novel, simple, accurate, and sensitive method to determine combinations such as HCTZ with ATEN, HCTZ with TELM, HCTZ with LOSA, HCTZ with VALS, HCTZ with OLME, and HCTZ with IBRE without altering the detection wave length and chromatographic conditions.

METHODS

The above said standard drugs were gifted from Hetero Labs Ltd., Hyderabad, India. All other chemicals used in this method were purchased from Merck Chemical Division Ltd., Mumbai. HPLC grade acetonitrile, water, methanol, and triethylamine were obtained from Merck Pharmaceuticals Private Ltd., Mumbai, India. Commercial tablets of the above said formulation were procured from local pharmacies.

Apparatus and instruments

RP-HPLC was done on an isocratic HPLC (Shimadzu LC-20AT prominence LC) with a LC-20AT pump, manual Rheodyne injector with a loop volume of 20 µl, variable wavelength Shimadzu SPD-20 A prominence ultraviolet (UV) detector, and Welchrom C_{18} Column (4.6 × 250 mm, 5 µm particle size). The HPLC system was set with "Spin chrome" software. An electronic balance (Shimadzu TX223L), digital PH meter (Systronics model - 802), a sonicator (spectral lab, model

UCB 40), and UV-Visible spectrophotometer (Systronics model-2203) were used in this analysis.

Preparation of pH 3.3 phosphate buffer

A 10 mM phosphate buffer was prepared by dissolving 6.056 g of potassium dihydrogen ortho phosphate in 445 ml of HPLC grade water. To this 55 ml of 0.1 M phosphoric acid was added and the pH was adjusted to 3.3.

Preparation of mobile phase

The above prepared buffer and acetonitrile were mixed in the proportion of 50: 50 v/v and were filtered through 0.45 µm nylon membrane filter and degassed by sonication

Preparation of stock standard solutions and calibration plot

Stock standard solutions containing 1.25, 0.25, 0.4, 0.5, 0.4, 0.8, and 1.5 mg/ml of HCTZ, ATEN, TELM, LOSA, VALS, OLME, and IRBE, respectively, were prepared by dissolving 12.5, 25, 40, 50, 40, 80, and 150 mg of each in the mobile phase in a 100 ml volumetric flask, respectively. It was then sonicated for 20 minutes and the eventual volume of the solutions was filled up to 100 ml with methanol to get stock standard solutions. To construct calibration plots, the stock standard solutions were diluted with the mobile phase to prepare working solutions to get various concentrations ranging from 2.5-12.5, 5-25, 8-40, 5-50, 8-40, 16-80, and 30-150 µg/ml for HCTZ, ATEN, TELM, LOSA, VALS, OLME, and IRBE, respectively.

Sample preparation

Twenty tablets of each sample Atenova-H (25 mg/tab ATEN), Cresar-H (40 mg/tab TELM), Hyzaar (50 mg LOSA), Valfect-H (40 mg VALS), Olmax-H (80 mg OLME), and Irovel-H (150 mg IRBE) and all these samples contains 12.5 mg/tab of HCTZ were weighed accurately and average weight was determined. The contents were ground into a fine powder and the powder equivalent to 100 milligrams of the drug was transferred in to a 100 ml of calibrated flask and dissolved in 70 ml of mobile phase with vigorous shaking and extracted 2 times with 10 ml portions of mobile phase and filtered through Whatman quantitative filter paper grade 41 into a 100 ml volumetric flask and diluted up to the mark with the same. From the above filtrate, 1 ml was further transferred to 100 ml volumetric flask and made up to the mark with mobile phase to obtain 100 µg/ml sample solution in each case. These prepared solutions were utilized as stock sample solutions throughout the work. Subsequent dilution of this solution was prepared with mobile phase to furnish the above-mentioned concentrations. The diluted solutions were analyzed under optimized chromatographic conditions employed and chromatogram is shown in Fig. 1.

Method optimization

When developing a novel method one of the most important goals is to accomplish a consistent reproducible separation. To acquire correct optimized HPLC conditions in the first instance in isocratic mode, copious trails were carried out by varying the different solvent mixtures such as acetonitrile, methanol, HPLC grade water, with or without buffers in different combinations, C_{18}, C_8 columns, and flow rates as well as pH of were tested. Based on the nature of the drugs eventually C_{18} column was chosen as a stationary phase. Trials of optimization were made by varying one parameter at a time, keeping all other parameters constant. Ultimately decorous resolution, short run time, excellent peak shape, minimal peak tailing, and good reproducibility results were identified when an analytical column of C_{18} and a mixture of mobile phase consisting phosphate buffer with pH 3.3 and acetonitrile in the ratio of 50:50 v/v, using a flow rate of 1mL/min are found to be suitable for most combinations. Optimized chromatographic conditions and system suitability parameters are shown in Table 1. UV overlain spectra of the ATEN and five separations with HCTZ are shown in Fig. 2.

Method validation

The developed method of analysis was validated in pursuance of the guidelines of ICH Q2 (R1) [22,23] to examine the parameters such as system suitability, linearity, specificity, precision, accuracy, and robustness, limit of detection (LOD), and limit of quantitation (LOQ).

RESULTS AND DISCUSSION

In the present study drug solutions of 10 µg/mL concentration solution of ATEN, HCTZ, TELM, LOSA, VALS, OLME, and IRBE were separately prepared with the mobile phase. All the seven drugs were scanned over the range of 200-400 nm in spectrum mode by applying UV spectrophotometer. By studying the overlain spectra, it was observed that all these four drugs showed optimum absorbance at 230 nm and were selected for further study. Accordingly, system suitability parameters such as retention time, resolution, the number of theoretical plates, efficiency/meter, and tailing factor of the peaks were computed for the optimized chromatographic conditions. As a result retention time of 2.431, 3.077, 3.230, 4.030, 5.243, 4.463, and 5.563 minutes, resolution of --, 3.354, 2.615, 2.755, 2.844, 3.675, and 2.057 for ATEN, HCTZ, TELM, LOSA, VALS, OLME, and IRBE were obtained. Moreover, the plate number of 11987, 12865, 13379, 14567, 15231, 15625, and 16058 was obtained for ATEN, HCTZ, TELM, LOSA, VALS, OLME, and IRBE and tailing factors of 1.164, 1.146, 1.128, 1.164, 1.143, 1.150, and 1.132 were obtained for ATEN, HCTZ, TELM, LOSA, VALS, OLME, and IRBE, respectively. As the results obtained were within the satisfactory limits, this method is apt for the separation and estimation of the above said drugs. Summary of validation parameters linearity, specificity, precision, accuracy, LOD, and LOQ is shown in Table 2.

Linearity

The purpose of this study is to verify that the detector response is directly proportional to the concentration of analyte in the sample. Linearity of developed method was determined by taking five different concentrations. The calibration curves for seven different drugs showed linearity over a concentration range of 5-25, 2.5-12.5, 8-40, 5-50, 8-40, 16-80, and 30-150 µg/mL for ATEN, HCTZ, TELM, LOSA, VALS, OLME, and IRBE, respectively. In ATENOVA - H 25 mg tablet

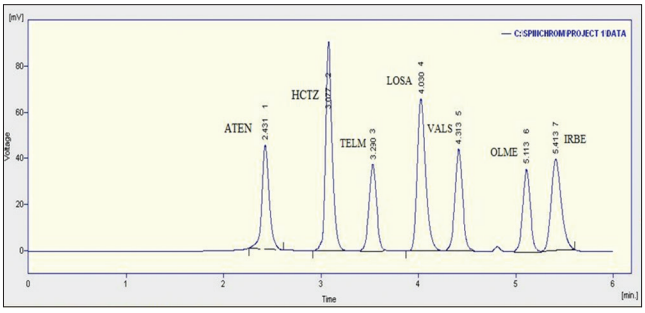

Fig. 1: A typical chromatogram showing the separation of atenolol and five angiotensin receptor blockade in the presence of hydrochlorothiazide mixture

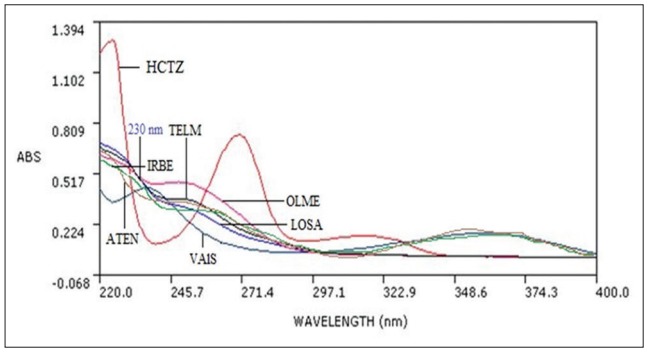

Fig. 2: Ultraviolet overlain spectra of the atenolol and five sartans with hydrochlorothiazide

Table 1: Optimized chromatographic conditions and system suitability parameters

Parameter	Chromatographic conditions for four sartans and hydrochlorothiazide						
Instrument	Shimadzu LC-20AT prominence LC						
Column	Welchrom C18 column (4.6×250 mm, 5 µm)						
Detector	Shimadzu SPD-20A prominence UV-VIS detector						
Mobile phase	10 mM phosphate buffer (pH 3.3): Acetonitrile 50:50, v/v						
Flow rate	1 mL/min						
wave length	UV at 230 nm						
Run time	6 minutes						
Temperature	Ambient temperature (25°C)						
Injection volume	20 µL						
	ATEN	**HCTZ**	**TELM**	**LOSA**	**VALS**	**OLME**	**IRBE**
Retention time (minute)	2.431	3.077	3.230	4.030	5.243	4.463	5.563
Th.Pl (efficiency)	11.987	12.865	13.379	14.567	15.231	15.625	16.058
Resolution	-	3.354	2.615	2.755	2.844	3.675	2.057
Tailing factors	1.164	1.146	1.128	1.164	1.143	1.150	1.132

LC: Liquid chromatography, UV-VIS: Ultraviolet-visible, ATEN: Atenolol, OLME: Olmesartan, HCTZ: Hydrochlorothiazide, TELM: Telmisartan, LOSA: Losartan, VALS: Valsartan, IRBE: Irbesartan

Table 2: Summary of validation parameters

Formulation (tablets)	Composition	Linearity (µg/mL)	LOD and LOQ (µg/mL)	Assay±SD (n=6)	Mean % recovery±SD (n=3)	Precision	
						Intra-day (n=6) (RSD%)	Interday (n=3) (RSD%)
ATENOVA-H	ATEN	5-25	0.331 and 1.02	99.95±1.24	99.92±0.67	0.343	0.342
	HCTZ	2.5-12.5	0.285 and 0.940	98.75±0.16	99.82±0.12	0.167	0.162
Cresar-H	TELM	8-40	0.565 and 1.86	99.97±1.12	100.09±0.71	0. 127	0.167
	HCTZ	2.5-12.5	0.298 and 0.980	98.14±0.32	100.05±0.16	0.132	0.324
Hyzaar	LOSA	5-50	0.197 and 0.650	99.87±0.12	99.98±0.42	0.467	0.472
	HCTZ	2.5-12.5	0.296 and 0.981	99.14±0.31	100.06±0.19	0.188	0.182
Valfect-H	VALS	8-40	0.731 and 2.414	99.88±1.14	100.02±0.16	0.342	0.324
	HCTZ	2.5-12.5	0.297 and 0.980	98.18±0.42	99.98±0.12	0.127	0.432
Olmax-H	OLME	16-80	1.198 and 3.953	99.99±1.12	100.45±0.40	0.432	0.654
	HCTZ	2.5-12.5	0.295 and 0.980	99.15±0.12	99.97±0.13	0.168	0.324
Irovel-H	IRBE	30-150	1.220 and 4.026	100.15±1.30	100.47±0.4	0.467	0.324
	HCTZ	2.5-12.5	0.296 and 0.980	99.59±0.12	99.96±0.15	0.213	0.435

LOD: Limit of detection, LOQ: Limit of quantitation, SD: Standard deviation, IRBE: Irbesartan, HCTZ: Hydrochlorothiazide, ATEN: Atenolol, OLME: Olmesartan, LOSA: Losartan, VALS: Valsartan, TELM: Telmisartan

5-25 µg/mL and 2.5-12.5 µg/mL for HCTZ; Cresar - H 40 mg tablet 8-40 µg/mL for TELM and 2.5-12.5 µg/mL for HCTZ; for Hyzaar 50 mg tablet 5-50 µg/mL for LOSA and 2.5-12.5 µg/mL for HCTZ; for Valent-H 80 mg tablet 16-80 µg/mL for VALS and 2.5-12.5 µg/mL for HCTZ; Olmax-H 40 mg tablet 8-40 µg/mL for OLME and 2.5-12.5 µg/mL for HCTZ; Irovel-H 150 mg tablet 30-150 µg/mL for IRBE 2.5-12.5 µg/mL for HCTZ and the correlation coefficients were calculated from the linear regression analysis and it was found to be above 0.9998 in all cases. The obtained results were satisfactory as there exist a significant correlation between concentrations of each drug and their peak areas. The r^2 (correlation coefficients) regression was found almost equal to one in all cases.

Specificity

The purpose of this study is to evaluated by determine the effect of excipients, additives present in the formulations interfered with the analysis or not. As per the ICH guidelines, to test the specificity of the developed method a combination of pure drug samples with proper excipients were injected to the system for quantifying each drug individually and also in combination of two drugs, namely, HCTZ with ATEN and HCTZ with TELM, HCTZ with LOSA, HCTZ with VALS, HCTZ with OLME, and HCTZ with IRBE. In the same way blank solution with only commonly utilized excipients and synthetic mixture solutions were also injected separately. Peak responses for analyte and the blank were compared with each appropriate drug. The present study shows that there is no commonly used excipients are interfering with the drug peaks. Thus, the method clearly proves to be specific for determining the above said drugs.

Precision

To check the reproducibility of the method, the precision of the analytical method was determined by using intraday and interday studies. Triplicate samples of standard quality were taken in varying concentration levels and estimated for the intraday and interday precision of the developed method. For repeatability and intermediate precision the relative standard deviation (RSD%) values for all the drugs were calculated and RSD% of all the combined drugs were shown <2% which explains that the current method is precise.

Accuracy

Accuracy of the present method was evaluated by combining the known quantity of pure standard drugs to pre analyzed samples at three different levels mostly 80%, 100%, and 120%. Then, the recovery levels were observed obviously. The above-mentioned solutions were again prepared and analyzed in triplicate carefully. The same procedure was followed for all the individual drugs and also for drug combinations such as HCTZ with ATEN and HCTZ with TELM, HCTZ with LOSA, HCTZ with VALS, HCTZ with OLME, and HCTZ with IRBE and RSD% calculated was also known to be <2% for each of the drugs. As per ICH guidelines the % recovery must be between 98% and 102%.

Robustness

Small, experimental conditions such as flow rate, detection wavelength, and mobile phase composition are deliberately changed. It was observed in fact no significant variations in these parameters indicating that the method is robust. The RSD% values obtained in all the cases are <2%, hence this method is robust. Table 3 summarizes the robustness, data.

Table 3: Robustness studies

Parameter	Used	ATEN	HCTZ	TELM	HCTZ	LOSA	HCTZ	VAL	HCTZ	OLME	HCTZ	IBRE	HCTZ
Flow rate (±2 mL/minute)	0.8 ml/min	99.63±0.59	99.78±0.68	99.91±1.01	98.94±0.48	98.96±0.99	99.83±0.29	99.98±0.29	100.0±0.53	98.88±1.00	98.76±0.48	99.96±1.03	99.63±0.79
	1.2 ml/min	97.99±0.47	98.96±0.27	100.0±0.99	99.74±0.43	99.86±1.23	96.84±0.86	100.3±0.97	99.84±0.43	100.0±1.88	98.77±0.35	98.04±0.93	99.87±0.35
Detection wavelength (±5 nm)	233 nm	98.77±0.35	98.98±1.02	99.87±1.66	99.63±0.59	99.62±1.64	98.97±0.78	100.0±0.89	99.78±0.68	100.1±1.67	100.0±0.44	99.74±0.68	100.0±0.89
	243 nm	96.42±1.29	100.0±1.00	98.98±1.02	97.04±0.96	100.0±1.00	99.98±1.02	99.86±1.65	98.67±1.41	98.79±0.63	96.86±1.99	99.63±1.93	99.74±0.67
Mobile phase composition (±5%)	45:55 v/v	99.86±1.65	98.67±1.41	98.94±0.98	99.72±1.05	99.83±1.62	98.72±0.98	99.80±0.31	98.96±0.27	98.71±0.86	100.0±0.53	98.99±0.24	99.63±0.59
	55:45 v/v	98.94±0.48	96.86±1.99	98.96±1.0	97.99±0.47	99.74±0.67	99.96±0.30	98.69±0.54	100.0±1.38	98.65±1.58	96.42±1.29	96.98±0.29	98.94±0.48

TELM: Telmisartan, LOSA: Losartan, OLME: Olmesartan, ATEN: Atenolol, HCTZ: Hydrochlorothiazide

LOD and LOQ

Sensitivity of the analytical method is evaluated by the LOD and LOQ. LOD=3.3 (SD)/S and LOQ=10 (SD)/S. The LOD and LOQ results for combined drugs such as ATEN with HCTZ, TELM with HCTZ, LOSA with HCTZ, VALS with HCTZ, OLME with HCTZ, and IBRE with HCTZ were found to be 0.331µg/mL and 1.026 µg/mL, 0.285 µg/mL and 0.940 µg/mL; 0.565 µg/mL and 1.86 µg/mL, 0.298 µg/mL and 0.980 µg/mL; 0.197 µg/mL and 0.650 µg/mL, 0.296 µg/mL and 0.981 µg/mL; 0.731 µg/mL and 2.414 µg/mL, 0.297 µg/mL and 0.980 µg/mL; 1.198 µg/mL and 3.953 µg/mL, 0.295 µg/mL and 0.980 µg/mL; 1.220 µg/mL and 4.026 µg/mL, and 0.296 µg/mL and 0.980 µg/mL, respectively. The lowest LOD and LOQ values showed that the method is more sensitive**.**

Assay

The developed method was finally applied for quantification of marketing formulation. Satisfactory results have been obtained. The mean assay values for ATEN with HCTZ, TELM with HCTZ, LOSA with HCTZ, VALS with HCTZ OLME with HCTZ, and IBRE with HCTZ combination of drugs were found to be 99.95±1.24 and 98.75±0.16; 99.97±1.12 and 98.14±0.32; 99.87±0.12 and 99.14±0.31; 99.88±1.14 and 98.18±0.42; 99.99±1.12 and 99.15±0.12; 100.15±1.30 and 99.59±0.12, respectively. The mean assay values were in good agreement with the label claim (Table 2). Hence, by the developed method all the specified drugs were recovered perfectly pharmaceutical dosage forms. Therefore, the method developed was found to be properly suitable for the estimation of the marketed formulations.

CONCLUSION

A simple, precise, accurate, and sensitive isocratic RP-HPLC method have been developed for simultaneous determination of HCTZ, ATEN, TELM, LOSA, VALS, OLME, and IRBE in bulk and combined tablet dosage forms. Statistical analysis lucidly proves that this method is fast, cost effective, highly efficient, and robust than the existing methods hitherto. In addition, to that, the method is free from interferences of the excipients and additives used in the preparation of the above-said combination formulations. This novel method separated all the above-said drugs in 4 minutes and total run time of just 6 minutes and it is also feasible for analysis of said angiotensin receptor blockades with HCTZ in their formulations in a single run without changing the mobile phase composition and chromatographic conditions. Hence, it is correct to conclude that it is effectively used for the application of all the above-said drugs individually or in binary combinations in quality control laboratories.

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