Simultaneous Determination of Sartans by High Performance Liquid Chromatography with Ultra Violet Detection

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ABSTRACT

Objective: A high performance liquid chromatographic method with ultra violet detection for simultaneous analysis of three sartans (Valsartan, Irbesartan and Telmisartan) has been developed for quality control.

Method and Results: Isocratic elution on a LiChrospher C18 column (250 × 4 mm, particle size 5 µm) at the temperature 30ºC with a mobile phase consisting of 10mM phosphate buffer: acetonitrile (65:35 v/v) at a flow rate 1.0 ml/min has been done. The column eluent was monitored with a UV detector at 225 nm. This allowed a rapid detection and identification as well as quantitation of the eluting peaks.

Method Validation: Calibration curves for all drugs were in the range of 5- 40 µg/ml and the linear regression coefficients were more than 0.995. Recovery rates for the sartans were in the range 96.5% to 103.1%. The limits of detection were calculated between 0.04- 0.06 µg/ ml. Also, the limits of quantification were 0.12- 0.16 µg/ml. Within-day and between-day coefficient of variation for all sartans at all concentrations in the range of 0.83 - 3.79% was calculated.

Conclusion: The procedure can provide a simple, sensitive and fast method for the quality control of the three sartans in bulk and tablets.

Key words: Drug analysis, Sartans, HPLC, Quality control, Hypertension, Heart failure.

INTRODUCTION

Nowadays hypertension is one of the major health problems worldwide. Angiotensin II is an octapeptide with strong hypertensive activity. It increases blood pressure as a result of shrinking effect on smooth muscular coat of vessels, releases aldosterone from an adrenal cortex and catecholamine from an adrenal medulla, which leads to retention of sodium ions and water in human body and an increase of circulating blood volume. Angiotensin II receptor antagonists (ARA II) selectively and specifically block the AT1 receptor of the renin angiotensin system by displacing angiotensin II from it.¹ Angiotensin antagonists are a major innovation for the treatment of hypertension and heart failure either alone or together with diuretics or recently with other antihypertensive drugs.² Medicines modifying renin-angiotensin system activity include: drugs reducing renin release (β-adrenolytic drugs), drugs blocking conversion of angiotensin I to angiotensin II - ACE (angiotensin converting enzyme) inhibitors and angiotensin receptor antagonists (ARB - angiotensin II receptor blockers), also known as sartans. They are better in preventing first occurrence of atrial fibrillation than beta-blocker (atenolol) or calcium antagonist (amlodipine) therapy.

Based on the recent literature, most of the publications deal with the determination of the following compounds in the ARB group – Valsartan, both for pharmaceutical
formulations\textsuperscript{3,7} and biological material: plasma\textsuperscript{3,8} and urine.\textsuperscript{9} It is normally determined by HPLC method with C18 column and UV-VIS detector\textsuperscript{25,36}, mass spectrometry (MS) detector\textsuperscript{4} or fluorescence detector.\textsuperscript{4} It is also determined spectrophotometrically\textsuperscript{9} and with TLC.\textsuperscript{10,12} Telmisartan is determined in human plasma,\textsuperscript{13,14} urine\textsuperscript{15,19} and binary tablets.\textsuperscript{20,21} It is determined by HPLC analysis using C18 column\textsuperscript{17,18,22} with mass sensitive detector,\textsuperscript{23,24} fluorescence,\textsuperscript{25} UV \textsuperscript{26,27} or DAD\textsuperscript{28,29} detector. Telmisartan is also determined simultaneously with hydrochlorothiazide by a spectrophotometric, densitometric and spectrofluorimetric analysis.\textsuperscript{30} Irbesartan in a biological material: plasma and urine\textsuperscript{31,35} is determined by an HPLC analysis with C18 column and DAD\textsuperscript{35,34}, MS detector\textsuperscript{35} or fluorescence detector,\textsuperscript{31} Irbesartan and hydrochlorothiazide in tablets are analyzed spectrophotometrically.\textsuperscript{36} But no analytical method has been reported yet for the simultaneous separation of all the three drugs namely Valsartan (VAL), Telmisartan (TEL) and Irbesartan (IRB). There are reports to determine the ARB that use solid phase extraction or other more complex time-consuming processing of the samples. That is why the aim of this research work was to develop a simple, rapid, precise, accurate, and economical RP-HPLC method, with a simple mobile phase for the simultaneous separation, identification and determination of the three compounds of the ARB group in bulk and pharmaceutical formulations. The proposed method was validated as per International Conference on Harmonization (ICH Q2, 2005) guidelines.\textsuperscript{37}

**MATERIAL AND METHODS**

**Reagents and Chemicals**

HPLC grade acetonitrile, methanol, potassium dihydrogen phosphate and ortho-phosphoric acid (Analytical grade) were obtained from Merck, Germany. Valsartan RS, Irbesartan RS and Telmisartan RS were used as standards and were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Apparatus and analytical conditions**

A Shimadzu HPLC system consisting of pump LC – 20 AD, vacuum degasser unit DGU – 20 A, column oven CTO-10AD and a UV/VIS variable detector SPD – 20 A was utilized for the analysis. The separation under reversed phase partition chromatographic conditions was carried out on a LiChrophosph C18 column (250×4 mm, particle size 5 µm). The equipment was controlled by a PC with installed Lab Solution software. The mobile phase was a 35:65 % v/v mixture of acetonitrile:phosphate buffer (10 mM, pH 6 ± 0.1, adjusted with ortho-phosphoric acid). The flow rate was 1.0 ml/min and the run time was 10 min. Before analysis both the mobile phase and sample solutions were degassed by the use of a sonicator and filtered through a 0.45 µm filter. The column temperature was maintained at 30°C and injection volume was 20 µl. The detection of the drug was monitored at 225 nm. The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in the standard solutions.

**Preparation of Stock Standard Solutions of VAL, IRB and TEL**

Individual stock standard solutions of the three drugs (200 µg/ml) were prepared by dissolving 10 mg of each drug in 50 ml of solvent A (methanol: water = 60:40). The methanol stock standards containing mixture of VAL (I), IRB (II) and TEL (III) were prepared by appropriately diluting the standard solutions in the range of 5–40 µg/ml using solvent A.

**Sample Preparation**

From formulations containing Valsartan (80 mg), Irbesartan (150 mg) and Telmisartan (80mg) was prepared a mixture of sample solutions. Twenty tablets of the three formulations were weighed, crushed separately to a fine homogenous powder and their mean mass was determined. Quantity equivalent to 80 mg of each formulation was accurately weighed and taken individually in a 20 ml volumetric flask. The powdered mixtures were dissolved in the methanol. The contents were sonicated for 15 min and the mixture was made up to 20 ml with methanol. It was filtered through 0.45 mm membrane syringe filter. The supernatants of the solutions were taken, mixed thoroughly and diluted with the solvent A. The final concentrations were 20 µg/ml for VAL, IRB and TEL, respectively. For the HPLC analysis was injected 20 µL of this solution and the peak areas were measured for the determination of VAL, IRB and TEL in tablet formulation.

**Method Validation**

The proposed method was validated under the established optimal chromatographic conditions. The validation as per ICH guidelines\textsuperscript{37} was carried out with respect to specificity, linearity, intra-day and inter-day precision, accuracy, and sensitivity (limit of quantitation (LOQ) and limit of detection (LOD)).

**Linearity**

The calibration graphs were obtained by injecting a series of standard solutions of each sartan separately.
into the HPLC system. The plotting mean chromatographic peak area against the concentration of each compound was made. Each solution was injected in triplicate and the mean peak area value was observed within the concentration range of 5 - 40 µg/ml for all sartans.

**Precision**

The system precision of the assays was investigated by performing five replicate analyses of the added standard samples at three different concentrations (10, 20 and 30 µg/ml) for each of the sartans on the same day and on three separate days. They were evaluated by relative standard deviations (RSD) of the peak areas of each analyte.

**Accuracy (recovery method)**

The accuracy of the HPLC method was tested by calculating the recovery of certain amounts of each of the sartans added separately at three different concentrations (10, 20 and 30 µg/ml) to samples representing the average weight of the corresponding sartans concentrations. The recoveries were also confirmed by determination of these drugs in samples containing 50, 100 and 150 % of sartans.

**Limiting values**

The limit of detection (LOD) was considered the lowest concentration of the analytes corresponding to three times the background noise or relationship signal-to-noise ratio 3:1.

The limit of quantification (LOQ) was defined as the lowest point of the calibration curve and fulfilled the requirement of LOQ signal-to-noise ratio of 10:1.

**RESULTS AND DISCUSSION**

**Selection of Mobile phase**

Different combinations of acetonitrile and phosphate buffer were tested and the optimum condition at acetonitrile-phosphate buffer 0.010 M (35:65 V/V) was reached.

**Effect of pH of mobile phase**

We studied the effect of varying the pH (5.5 - 6.5). Moreover, the stability of sartans is low in the alkaline media. We found out that the best separation results were achieved at pH 6.

**Selection of flow rate and column temperature**

Increasing of the column temperature from 25°C to 40°C led to a decrease in the total time required for the separation process with decrease of peak broadening and increase in sensitivity. The optimum column temperature was at 30°C. Also, increasing the flow rate from 1 ml/min to 1.5 ml/min showed a similar decrease in the retention time. The optimum flow rate was 1.0 ml/min. The obtained chromatogram of the selected sartans with a rapid separation at different retention times is shown in Figure 1. The proposed chromatographic conditions indicate that the method is selective and could be applied for simultaneous identification and quantification of the sartans.

**Method validation**

**Specificity and selectivity**

The specificity of the HPLC method was established by analyzing standard drug and sample solutions. The retention times of VAL, IRB and TEL were confirmed by comparing the retention time with that of the standard (Figure 1). The selectivity of the method there is no interference between the matrix of blank sample and the drug sample.

**Linearity**

The linear calibration curves for VAL, IRB and TEL were constructed with five concentration levels each under the experimental conditions described above. The calibration curves of each drug substance were subject to regression analysis to calculate the regression equations and the correlation coefficients. Table 1 shows the regression equations of the selected sartans. In the regression equation; $y = ax + b$, “x” is for the concentration of the standard sartans, ”y” is for the peak area, “a” is the intercept of the straight line with y-axis and “b” is the slope of the line. The $R^2$ in Table 1 refers to the correlation coefficient of the equation. All the standard sartans showed good linearity ($R^2 > 0.995$) in a relatively wide concentration range, adequate for the analytical method. Calibration plot data slope (a), intercept (b), and correlation coefficients ($R^2$) are given in Table 1.

![Figure 1: Chromatogram referring to the separation of selected sartans. Peak 1: Valsartan (1.86 min), Peak 2: Irbesartan (3.18 min), Peak 3: Telmisartan (8.96 min).](image-url)
Limits of quantitation and Limits of detection

The determined values of LOD and LOQ for the selected sartans in the proposed method are shown in Table 2. In this method, LOD for VAL and TEL was 0.04 µg/ml and for IRB was 0.06µg/ ml. LOQ for VAL and TEL was 0.12 µg/ml and for IRB was 0.16 µg/ ml.

Accuracy /Recovery

Accuracy of the proposed method was determined using recovery studies. The recovery study results of the selected sartans ranged from 96.5% to 103.1% using solution of sample preparation. The coefficients of variation for this technique were lower than 5 %. Results are reported in Table 3.

Precision

The precision of the quantitative method is the degree of agreement among the individual test results, of the repeated procedure to multiple samplings. It is measured by repeatedly injecting a ready-made sample and is expressed as coefficient of variation of the results. Within-day (n = 5) and between-day (n = 3) precision presented coefficients of variation and relative errors lower than 5%. These results are presented in Table 4.

CONCLUSION

A simple isocratic RP-HPLC method with UV detection has been developed for simultaneous determination of VAL, TEL and IRB. The method was validated for accuracy, precision, specificity and linearity. The run time was relatively short (10 min), which enabled rapid quantification of many samples in routine and quality control analysis of tablets. It is suitable for analysis of antihypertensive agents in their formulations in a single isocratic run. HPLC with UV detection becomes the
most available apparatus and is a low cost instrument in comparison with HPLC coupling with mass spectrometry and capillary electrophoresis.

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CONFLICT OF INTEREST
Authors have no conflicts of interest to declare.

ABBREVIATIONS USED
ARA II: angiotensin II receptor antagonist; ACE: Angiotensin converting enzyme; ARB: Angiotensin II receptor blocker; HPLC: High performance liquid chromatography; UV: ultraviolet; MS: Mass spectrometry; TLC: Thin layer chromatography; DAD: Diode array detector; VAL: Valsartan; IRB: Irbesartan; TEL: Telmisartan; ICH: International Conference on Harmonization; LOQ: Limit of quantitation; LOD: Limit of detection; RSD: Relative standard deviation.

REFERENCES
A validated method was developed for simultaneous analysis of three sartans (Valsartan, Irbesartan and Telmisartan) in tablet dosage form by RP-HPLC method.

- A mixture of acetonitrile: phosphate buffer 10mM, pH 6 adjusted using 0.1 mol/l o-H3PO4 in 35:65 v/v ratio was used as mobile phase.
- The retention times of Valsartan, Irbesartan and Telmisartan were found to be Peak 1: Valsartan (1.86 min), Peak 2: Irbesartan (3.18 min), Peak 3: Telmisartan (8.96 min).
- The developed method was validated as per ICH guidelines.
- The procedure can provide a simple, sensitive and fast method for the quality control of the three sartans in bulk and tablets.