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Development & Validation of HPLC Method for Analysis of Some Antihypertensive Agents in their Pharmaceutical Dosage Forms

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Abstract:
A simple, precise, fast and gradient, high performance liquid chromatographic (HPLC) method was developed and validated for the determination of Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide in solid dosage forms. The quantitative determination of analyte(s) was performed on a PUROSPHERE STAR RP 18e analytical column (250×4.6 mm) with 0.2 %v/v TEA buffer (pH: 3.0): ACN as mobile phase, at a flow rate of 1.0 mL min^{-1}. Detection was made by extracting PDA spectra at 215 nm respectively. During method validation, parameters such as precision, linearity, stability, Robustness, Ruggedness and specificity were evaluated, which remained within acceptable limits. The method has been successfully applied to assess the assay of solid dosage formulations.

Keywords: *Liquid chromatography; Aliskiren; Ramipril; Valsartan; Hydrochlorothiazide; Solid dosage formulations; Validation; simultaneous determination*

Introduction:

Hypertension or **high blood pressure** is a chronic medical condition in which the blood pressure in the arteries is elevated. It is classified as either primary (essential) or secondary. About 90-95 % of cases are termed "primary hypertension", which refers to high blood pressure for which no medical cause can be found the remaining 5-10 % of cases (Secondary hypertension) are caused by another conditions that affect the kidneys, arteries, heart, or endocrine system. Blood pressure is classified based on two types of measurements, the systolic and diastolic blood pressures expressed as a ratio such as '120 over 80' (120/80) mmHg. Systolic blood pressure is the blood pressure in vessels during a heart beat. Diastolic blood pressure is the pressure between heartbeats.

Aliskiren (Figure1a), a highly potent and selective inhibitor of human renin in vitro, and in vivo; once-daily oral doses of Aliskiren inhibit renin and lower blood pressure in sodium-depleted marmosets and hypertensive human patients. Aliskiren represents the first in a novel class of renin inhibitors with the potential for treatment of hypertension and related cardiovascular diseases. Aliskiren administered both orally or intravenously. Aliskiren is also available as combination therapy with Hydrochlorothiazide.

Ramipril (**Figure1b**) is an angiotensin-converting enzyme (ACE) inhibitor, used to treat hypertension and congestive heart failure. ACE inhibitors lower the production of angiotensin II, therefore relaxing arterial muscles while at the same time enlarging the arteries, allowing the heart to pump blood more easily, and increasing blood flow due to more blood being pumped into and through larger passageways. Ramipril is a prodrug and is converted to the active metabolite ramiprilat by liver esterase enzymes.

Valsartan **(Figure1c)** is in a class of drugs called angiogenesis II receptor antagonists. This medicine works by preventing constriction (narrowing) of blood vessels (veins and arteries). Valsartan is used to treat high blood pressure (hypertension), congestive heart failure and to reduce cardiovascular death in patients. Valsartan is available as tablets for oral administration, containing 40 mg, 80 mg, 160 mg or 320 mg of valsartan. Valsartan may cause hypotension if it is taken with other heart medication.

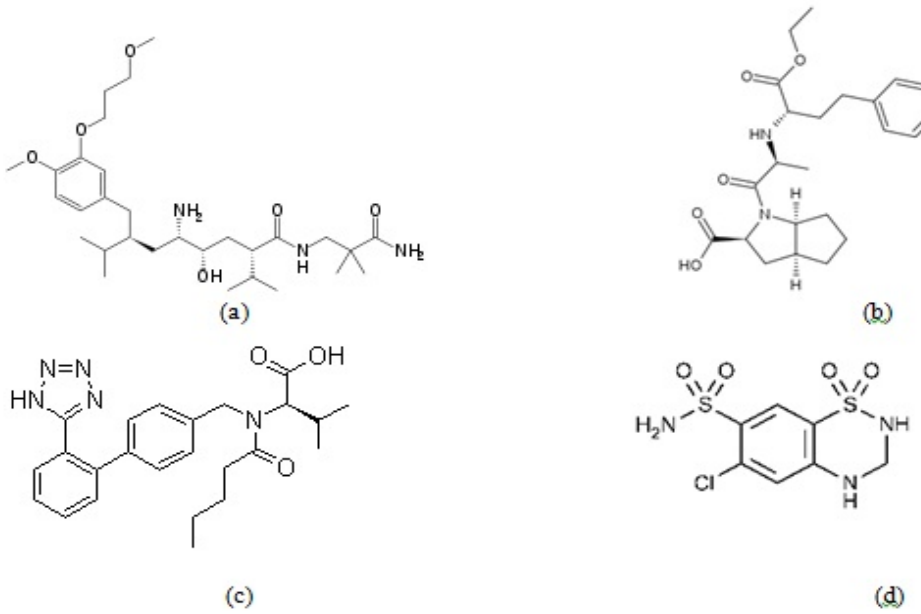

Figure 1: Chemical structures of (a) Aliskiren, (b) Ramipril (c) Valsartan and **(**d)Hydrochlorothiazide

Hydrochlorothiazide (Figure1d) is a first line diuretic drug of the thiazide class that acts by inhibiting the kidneys' ability to retain water. This reduces the volume of the blood, decreasing blood return to the heart. Hydrochlorothiazide is used to treat excessive fluid accumulation and swelling (edema) of the body caused by heart failure, cirrhosis, chronic kidney failure, corticosteroid medications, and nephrotic syndrome. The usual adult dose for hypertension is 12.5 to 50 mg once daily. The usual adult dose for treating edema is 25-100 mg once daily or in divided doses. Blood sugar levels can be elevated by hydrochlorothiazide, necessitating adjustment in the doses of medications that are used for treating diabetes.

The safety and efficacy of drug therapy can be ensured using a validated analytical method to assess the quality of pharmaceutical products as it has been considered suitable for their intended purpose, like quantitation of active ingredients (assay).

Since validated methods are applied routinely, some essential aspects must be observed during the technique development to avoid an excessive waste of financial resource. The mobile phase is an important factor to be considered during development of the HPLC method. Thus, solvents that have a low price and extend the column life are generally chosen. Also the choice of column should be such that it should be rugged, easily available; batch variation should be less and can sustain a longer life.

Furthermore, the application of the method is relevant because it is fast and determines the range of linearity. The present work reports the development and validation of a method that can be applied for the determination of Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide in individual dosage form and in combination. Furthermore the method has stability indicating capability.

Experimental:

Chemicals & Reagents

All solvents were of HPLC grade and all reagents were of analytical grade.

Triethylamine, *ortho*-phosphoric acid and hydrogen peroxide were obtained from SD Fine Chemicals (India). Acetonitrile, Hydrochloric acid, sodium hydroxide was obtained from Rankem (India). Water was purified with Milli-Q Plus, Millipore System (USA). All solvents and solutions were filtered through a membrane filter (Millipore Millex -HV filter units, 0.45 µm pore size; nylon) and degassed before use. All solutions were profiteered before injecting into HPLC system using Millipore milex hydrophilic PTFE unit filter of 0.45 µm pore size.

Instrumentation and Analytical Conditions

The HPLC method development and validation procedures was performed on Waters Alliance HPLC system (waters 2695 separation module), equipped with a photo diode array detector (Waters 2996 photo diode array detector). Data integration was performed using Empower-1 software. The analytical column was a normal phase 18e (250*4.6 mm, 5 µm particle size) (PUROSPHERE STAR RP). All analysis was carried out at a temperature of 40±2 °C under gradient conditions. The mobile phase consisted of a mixture of 0.2 % v/v Triethylamine buffer (pH 3.0, adjusted with diluted ortho-phosphoric acid): Acetonitrile. The flow rate was 1.0 mL/min, the volume of injection was 20 µL, all chromatograms were monitored in 200-400 nm range using Photo diode detector and the detection was made by extracting chromatogram at 215 nm in table 1.

Table 1: Concentration

Time	Buffer conc.	ACN conc.
0	90	10
4	85	15
10	30	70
18	90	10
25	90	10

Sample Preparations

Aliskirene, Ramipril, Valsartan and Hydrochlorothiazide were taken respectively 50 mg (99.7 %), 50 mg (99.8 %) 25 mg (99.8 %) and 25 mg (99.8 %) accurately weighed and transferred to a 50 mL volumetric flask and to this about 3 mL of ACN was added, mixture was sonicated for 5 min and then diluent (ACN:Water 1:1) was added, resulting mixture was sonicated . 5 mL of each solution was diluted to 50 mL with the diluent (Water:ACN 1:1) to get final concentration of 50, 50, 25 and 25 µg mL^{-1} for Aliskirene Ramipril, Valsartan and Hydrochlorothiazide respectively. Resulting solution was filtered through 0.45µm pore size PTFE filter units before use.

Method Development

The chromatographic conditions were adjusted in order to provide a good assay performance. Mobile phase selection was based on peak parameters (tailing) and run time.

Method Validation:

The method applied for the determination of Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide in pharmaceuticals was validated according to the International Conference on Harmonisation guidelines for analytical procedures validation. [29]

Linearity

The linearity was evaluated by linear regression analysis using the least square regression method. The calibration curve was obtained with fifteen concentrations of standard solution (20-120 % of nominal concentrations with 10 % increment) for the chromatographic method.

Precision

Six injections of the standard mixture were analyzed for the determination of system precision. Similarly six solutions of the individual standards were prepared and assayed for the determination of method precision.

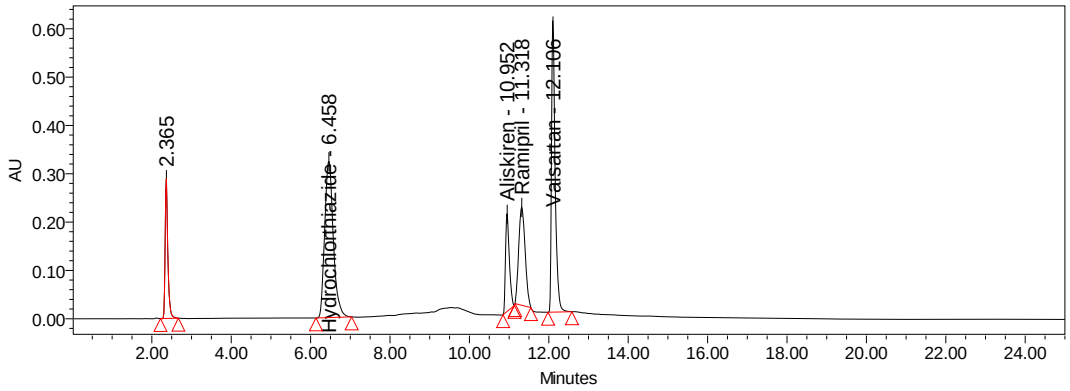

Figure 2: shows a typical chromatogram obtained from the analysis of a standard mixture using the proposed method.

Specificity

The specificity was determined by analyzing system blank, diluent and different placebos.As shown in this figure, peaks represent Aliskiren (Run Time 10.952 minute and Tailing 1.5), Ramipril (Run Time 11.318 minute and Tailing 1.1), Valsartan (Run Time 12.106 minute and Tailing 1.5) and Hydrochlorothiazide (Run Time 6.458 minute and Tailing 1.2) respectively.

The possible interferences were analyzed by the peak purity, which was calculated using Empower-1 software.

For degradation studies, samples were subjected to acidic, alkaline and oxidative degradation the resulting solutions were analyzed using proposed method.

Robustness

For the HPLC method, the robustness was determined by the analysis of the samples under a variety of conditions making small changes in the buffer pH (2.8 and 3.0), in the percentage of mobile phase component- ±2.0 % (Buffer: ACN), in the flow rate (0.9 and 1.1 mL min^{-1}), in the temperature conditions (35 ºC and 45 ºC) and by changing the wavelength of detection (210 and 220 nm).

Stability

The sample was analyzed for more than 27 hrs at room temperature i.e. at 25 ºC and found stable.

Results and Discussion:

Linearity

The calibration curve of analytes was assessed by plotting concentration versus peak area The R^2 values was found to be 0.9993, 0.9927, 0.9997 and 0.9997 for Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide respectively.

The linear range obtained for the procedure applied to formulations by HPLC allows one to assay of active pharmaceutical ingredient containing Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide in individual formulation.

Precision and accuracy

The calculated results for accuracy and precision in table 2.

Table 2: Precision data

Parameter	Aliskiren	Ramipril	Valsartan	Hydrochlor othiazide
System Precision (% RSD)	0.28	1.14	0.41	0.30
Method Precision (% RSD)	1.87	0.92	0.43	0.26

Parameter	Normal	Variation	Aliskiren	Ramipril	Valsartan	Hydrochlorothiazide
			% RSD			
pH of buffer (pH unit)	3.0	2.8	0.65	1.81	0.82	1.60
		3.2	1.34	1.61	0.56	1.88
Flow rate (mL min^{-1})	1.0	0.9	1.14	1.25	1.33	0.88
		1.1	1.75	1.12	1.53	1.46
Column Temperature (ºC)	40	35	1.84	1.15	0.82	1.49
		45	1.79	1.57	0.38	1.19
Wavelength (nm)	215	210	0.76	1.28	1.08	0.91
		220	1.35	1.58	1.71	1.88

Mobile Phase-

Parameter	Aliskiren	Ramipril	Valsartan	Hydrochlorothiazide
Mobile Phase (-2 %) (% RSD)	1.31	1.07	0.39	1.79
Mobile Phase (+2 %) (% RSD)	1.54	1.70	0.65	1.42

Specificity

For the proposed HPLC method, no interference from the matrix and excipients was found in the placebo of the tablets.

The chromatograms show that peaks are pure, satisfy system suitability criteria and drug not degraded more than 30 %.

Robustness

The HPLC method demonstrated robustness for all evaluated parameters.Robustness of the method for determination of Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide.

Stability

The drug stability was determined up to 27 hours. The cumulative % RSD value for Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide was found to be 0.9 %, 1.6 %,1.1% and 1.2 % respectively.

Conclusion:

The proposed HPLC method enables a fast quantitative determination of Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide in individual formulation or in combinations. It is of practical utility because all the molecules are available in individual formulation and a combination of Aliskiren and Hydrochlorothiazide is available. The application of this method in routine analysis can be justified since easy sample preparation steps are involved with simple reagents and solvents were used experimentally. The validation demonstrated that the procedure is suitable for the intended purpose because the method was considered linear, precise, robust, rugged and specific and can be employed in quality control of pharmaceuticals containing Aliskiren, Ramipril, Valsartan and Hydrochlorothiazide. It is advisable to prepare individual standard solutions in case when single analyte is present in dosage form. As the run time is only 25 min this method is very economical in regular use.

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