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METHOD DEVELOPMENT AND VALIDATION OF A COMBINATION OF VALSARTAN AND POLYTHIAZIDE AS ANTIHYPERTENSIVE AGENTS

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ABSTRACT

Valsartan (VAL) and Polythiazide (PTZ) combination is a crucial therapeutic advancement in the management of hypertension, necessitating precise analytical methods for ensuring quality. A specific, fast RP-HPLC method was developed and validated for co-estimation of VAL and PTZ on a Kromasil C-8 column (150×4.6 mm, 5µm). Maximum separation was attained with gradient elution by the use of ammonium dihydrogen phosphate buffer (pH 3.0) and methanol: acetonitrile (50:50) as mobile phases at the flow rates of 1.2 mL/min and detection wavelength 265 nm. The method had an excellent linearity ($R^2 > 0.999$) in ranges 8-12 µg/mL for PTZ and 32-48 µg/mL for VAL. Method validation revealed good precision (RSD ≤ 0.39%), accuracy (recovery 98.40-100.25%), and robustness. LOD and LOQ were 0.15 µg/mL and 0.45 µg/mL for PTZ, and 0.25 µg/mL and 0.75 µg/mL for VAL, respectively. The short analysis time (10 min) of the method, easy mobile phase preparation, and extensive validation of the method make it ideal for routine quality control analysis of this valuable antihypertensive combination, with the advantage of quicker analysis and better sensitivity than the prevailing methods.

Keywords: Valsartan, Polythiazide, RP-HPLC, Method Validation, Simultaneous Determination, Antihypertensive Agents, Quality Control.

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INTRODUCTION

Hypertension is still a major cause of cardiovascular morbidity and mortality globally, with an estimated 1.28 billion adults worldwide. The combination of VAL and PTZ is a new therapeutic strategy using two complementary modes of action to achieve better blood pressure control. VAL, an angiotensin II receptor blocker(ARB), and PTZ, a thiazide diuretic, act synergistically to offer better antihypertensive effects than monotherapy.[1] Current studies prove this combination effective in decreasing cardiovascular events and deaths in patients with heart failure, contributing to Sustainable Development Goal 3 (Good Health and Well-being) directly through better management of non-communicable diseases.[2] Recent advances in analytical methods for valsartan estimation have resulted in the development of versatile techniques specific to different matrices and formulations.[3,4]A number of analytical techniques have been detailed in current literature for VAL and PTZ analysis, together with other drugs or independently, and no single technique has been constructed for the estimation of these two drugs in combination.[5,6,7,8,9,10,11,12,13] The current research gap is addressed in this investigation by designing and validating an efficient, selective RP-HPLC method for the simultaneous estimation of VAL and PTZ in drug products, which will make a contribution to medication safety, efficacy, and finally improved health outcomes. The current study aims to prepare and authenticate a reliable, rapid, and simple RP-HPLC technique for the concurrent determination of VAL and PTZ in dosage forms, considering the present demand for quality control strategies in combination drug products.

EXPERIMENTAL

Instrumentation

Separation was conducted on an Agilent 1260 Infinity II HPLC with a UV-visible detector and EZ Chrome data-handling system. Analysis was performed on a Kromasil C-8 column (150×4.6 mm, 5µm) at

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room temperature (25°C ± 2°C). Other equipment used included an ultrasonic cleaner, a digital pH meter, an electronic balance (Redbeg), and 0.45 µm nylon syringe filters.[14]

Chemicals and Reagents

The active pharmaceutical ingredients (APIs) of Valsartan and Polythiazide were provided as gift samples from Biophore India Pharmaceutical Pvt. Ltd. and Enomark Biotech India. Ammonium dihydrogen phosphate, phosphoric acid, acetonitrile, methanol, Mille-Q water, and purified water of HPLC Grade were bought from the local pharmacy.

Chromatographic Conditions

Chromatographic separation was performed on a Kromasil C-8 column due to its most efficient selectivity and ability to separate both the analytes effectively. The mobile phase system had two components: Mobile phase A was prepared by dissolving 2.0 g of ammonium dihydrogen phosphate in water and diluting it to 1000 mL, and adjusting to pH 3.0 using phosphoric acid, which supplied adequate ionic strength and pH regulation for the reproducible retention. Mobile phase B consisted of a 50:50 (v/v) mixture of acetonitrile and methanol, selected following extensive optimization studies to produce the best peak shape and resolution. This two-component solvent system showed better chromatographic performance than traditional single organic phase systems, with good peak symmetry and baseline resolution for both Valsartan and Polythiazide.

The choice of these mobile phase constituents was based on their polarity, solution stability, and compatibility with the target analytes. Chromatographic separation was carried out under the optimum conditions with the column kept at room temperature (25°C ± 2°C). The mobile phase was supplied at a flow rate of 1.2 mL/min, which ensured efficient peak separation and acceptable back pressure. Detection was done with a UV detector operated at 265 nm, the maximum absorption wavelength for both analytes as revealed by spectral analysis. A careful selection of injection volume of 10 µL was made to provide sufficient sensitivity while preserving peak symmetry and column efficiency. The overall chromatographic run time, including between-injection equilibration, was established at 10 minutes to enable high sample throughput while allowing for complete elution of all components and return to baseline.

Preparation of Buffer Solution

Dissolve 2.0 g of ammonium dihydrogen phosphate in water and dilute with water to make 1000 ml, and adjust with phosphoric acid to a pH of 3.0.[15]

Preparation of Diluent Solution

50% of Buffer solution, 25% of methanol, and 25% of acetonitrile were mixed well to obtain a 50:25:25% (v/v) mixture of Buffer Solution, methanol, and Acetonitrile. This mixture was thoroughly mixed and then sonicated in an ultrasonic bath for 5 minutes before being used for analysis. The solution is labeled and must be used within 5 days of the preparation date.

Blank Preparation

Diluent used as a blank for this experiment.

Standard Stock Solution of Valsartan

Weigh exactly and transfer about 40 mg of Valsartan Sodium API into a 100 ml flask. Add approximately 50 ml of methanol to dissolve the API, and then adjust the volume with methanol to fill the flask.

Standard Stock Solution of Polythiazide

Weigh exactly and transfer about 10 mg of polythiazide API into a 100 ml flask. Add approximately 50 ml of methanol to dissolve it, and then fill the flask to the desired volume with methanol.

Working Solution Preparation from the Standard Solution of Valsartan

5.0 ml of the standard stock solution of valsartan sodium was transferred into a 50 ml volumetric flask. The solution was then brought up to the mark with the diluent and thoroughly mixed.

Working Solution Preparation from the Standard Solution of Polythiazide
Take 5.0 mL of the standard stock solution of polythiazide, transfer it into a 50 mL volumetric flask. Fill the flask with diluent until it reaches the mark, then mix thoroughly.

Standard Solution Preparation for Simultaneous Estimation of Telmisartan and Polythiazide
Each 5.0 ml of the standard stock solution of valsartan and the standard stock solution of polythiazide are transferred into a 50 ml volumetric flask. The solution is then brought up to the mark with the diluent and mixed thoroughly.

Sample Solution Preparation
For pharmaceutical sample preparation, a weight of twenty tablets of the combination drug product consisting of Valsartan (160 mg) and Polythiazide (1 mg) was measured accurately and transferred to a mortar, and then powdered finely using a pestle. The mean weight was determined, and powder equivalent to a single tablet was weighed precisely and transferred to a 100 mL amber volumetric flask.[16-17] The solution cooled to room temperature and was then diluted to mark with diluent. Upon well-mixing, the solution was filtered via a 0.45 µm nylon membrane filter, pouring away the first few milliliters of filtrate. The prepared stock solution had 1600 µg/mL of VAL and 10 µg/mL of PTZ.[18]

System Suitability and Analytical Parameters
System suitability tests were conducted to ensure the performance of the chromatographic system for analysis. The acceptance was set according to ICH guidelines and comprised: theoretical plates count (N) of at least 2000 for VAL and PTZ peaks, tailing factor not exceeding 2.0, VAL-PTZ peak resolution more than 5.0, and relative standard deviation (RSD) of peak areas obtained from five repetitive injections not above 2.0%.[19] The capacity factor (k') was kept within the range 2-10, and the peak purity index at not less than 0.999. System performance was checked regularly in analysis by injecting the standard solution at the start and end of each series of analyses, with a standard injection following every ten injections of samples. [20] For the determination of LOD and LOQ, a signal-to-noise ratio method was used based on the equations $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$, where σ is the standard deviation of the response and S is the slope of the calibration curve. Six solutions of decreasing concentrations (0.05-2 µg/mL) were prepared and determined six times each. The concentration was plotted versus signal-to-noise ratio, and the standard deviation of y-intercepts computed. Slope was obtained from the linearity plot, and equations were used to calculate LOD and LOQ values. Experimentally validated by determining LOD and LOQ values by estimating concentrations of solutions with these values to check for efficient detection and quantification.[21]

RESULTS AND DISCUSSION

Chromatography optimization

The chromatographic conditions were optimized to obtain the optimum resolution and peak shape for VAL and PTZ. Various mobile phases with acetonitrile and methanol in ammonium phosphate buffer were studied. For the best separation of the drug under gradient conditions, various combinations of mobile phase were tried with the usual solvents such as ammonium phosphate buffer, methanol, and acetonitrile with or without various buffers at pH levels on the C18 stationary phase. The combination of buffer solution, methanol, and acetonitrile in a gradient ratio was found to be the most appropriate among all the combinations because the obtained chromatographic peaks were more resolved, defined, and nearly tailing-free. A mobile phase flow rate of 1.2 ml/min was found to be appropriate within the range of 0.5-1.5 ml/min studied. Therefore, the gradient method was selected as optimal for obtaining well-defined and resolved peaks with mean retention times of 2.21 and 6.58 for the standard solutions polythiazide and valsartan, respectively, as shown in Fig.-1.

Validation of the Analytical Method
Analytical method validation is the process of determining, through laboratory studies, that the performance characteristics of the method meet the requirements for its intended analytical applications. The characteristics that are studied during method validation include system suitability, accuracy (recovery), precision, specificity, linearity of detector response, range of analyte concentration, and robustness.

System Suitability and Specificity (Interference study)

Specificity ensures the identification of an analyte. Specificity of the method was defined by observing the interference of the blank, placebo, standard solution, sample solution, and individual solution of the component, and the proposed method was evaluated by the system suitability parameter, and the retention time of the drugs was found satisfactory under the individual solution of drugs and represented by the system suitability parameter shown in Table-1.

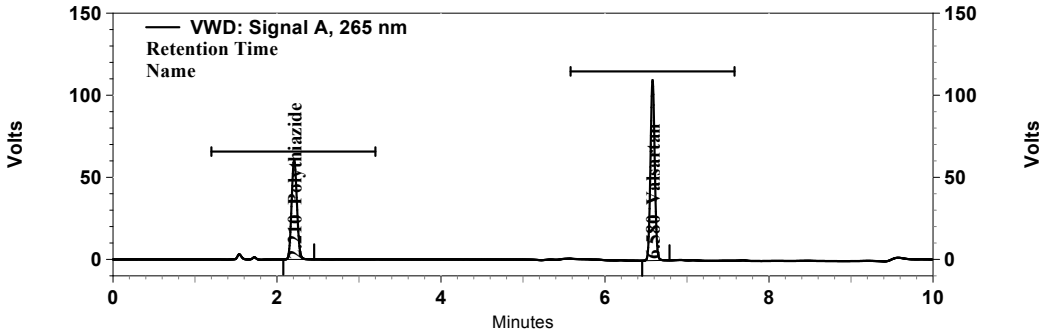

Fig.-1: Chromatogram of Standard Solution of Polythiazide and Valsartan, respectively at Optimized HPLC Conditions

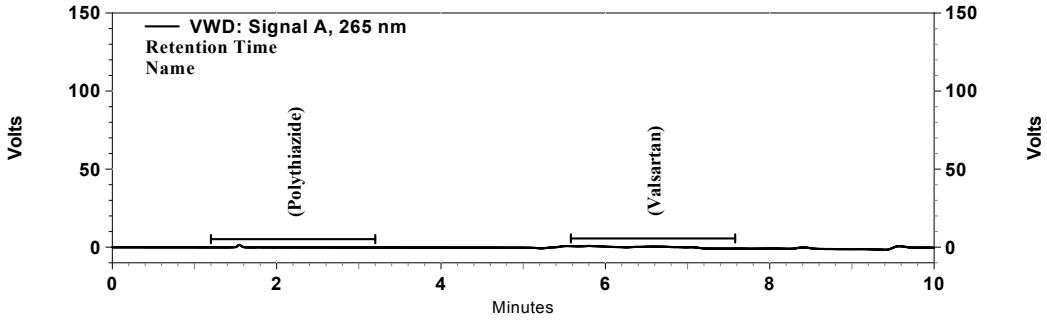

Fig.-2: Chromatogram of Blank Solution at Validated HPLC Conditions

Table-1: System Suitability Parameter of Both Drugs, Valsartan, and Polythiazide

Ingredient	Retention Time	RSD	Asymmetry factor	Theoretical plates	Resolution
Polythiazide	2.21	0.23	1.01	15263	-
Valsartan	6.58	0.31	0.98	22145	10.31

Linearity & Range

Standard stock solutions of the drugs were diluted to prepare linearity solutions from the standard stock solution in the concentration range of 80-120% of the standard concentration. i.e., (80%, 90%, 100%, 110%, and 120%) of the specific concentration of polythiazide and valsartan. The calibration curve of the analytical method was measured; the peak areas were proportional to the concentrations of analytes. It was showing suitable linearity in a range of 8-12 µg/ml for PTZ and 32-48 µg/ml for VAL, respectively.

Table-2: Linearity Study for Valsartan and Polythiazide

Polythiazide (PT)		Valsartan (VAL)	
Concentration	Area (Response)	Concentration	Area (Response)
8 µg/ml	201249	32 µg/ml	425471
9 µg/ml	226403	36 µg/ml	478659
10 µg/ml	251564	40 µg/ml	531849
11 µg/ml	216711	44 µg/ml	585024
12 µg/ml	311862	48 µg/ml	638211

Accuracy (Recovery)

The accuracy of an analytical method validation is the closeness of the test results obtained by that procedure to a true known value. The accuracy of the developed method was determined by spiking the combination products with API standards of compounds under study. The difference between the spiked sample result and the non-spiked sample result was calculated as a percentage of the known added spike concentration. For this purpose, the prepared combination product that contained two compounds was used. The standards were added to the samples at 3 concentrations, 50%, 100%, and 150% of the assay concentrations, and injected in triplicate. The % recovery of each added working standard was regarded as the accuracy (Table-3). The recoveries of all compounds were within the specified guidelines (ICH) of 95.0-105.0%.

Table-3: Recovery Study for the Determination of Polythiazide (PT) and Valsartan (VAL) by the Proposed Method

Sample Name	Sample concentration (µg/ mL)	Average of concentration Found (µg/ mL)	Assay %	Recovery %	RSD %	Average of Recovery and RSD
PT	8	8.01	50.07	100.13	0.38	99.99 % 0.39 %
	10	9.96	99.60	99.60	0.49	
	12	12.03	150.38	100.25	0.31	
VAL	20	19.68	49.2	98.40	0.53	99.36 % 0.31 %
	40	39.82	99.50	99.55	0.21	
	60	60.07	150.18	100.12	0.19	

Solution Stability

The Sample solution was prepared as directed in the methodology. The solutions were injected at initially to 24 hrs. as per the analytical method.

Repeatability (Precision) and Intermediate Precision

Precision was studied by measuring intraday and interday variability. A study was carried out by injecting six replicates of different sample solutions as per the methodology, and the % result of the peak areas was determined and reported in Table-4.

Table-4: Precision studies of Valsartan and Polythiazide

Parameter	Repeatability		Intermediate Precision	
Ingredient	Polythiazide	Valsartan	Polythiazide	Valsartan
Sample-1	254498	523961	255109	519971
Sample-2	256206	529157	256077	520362
Sample-3	256127	528801	255441	521163
Sample-4	256351	528968	255384	524179
Sample-5	256245	529204	255619	518925
Sample-6	255917	528873	254953	519658
Mean	255891	528161	255431	520710
SD	697.56	2063.38	396.77	1854.69
% RSD	0.27	0.39	0.16	0.36

Robustness

The robustness was studied by evaluating the effect of small but deliberate variations in the chromatographic conditions. The conditions studied were flow rate (± 0.02 mL min-1), wavelength (± 5 nm), and column oven temperature (± 5.0°C). % RSD for all the robustness studies is shown in Table-5.

The newly developed method offers several significant advantages over previously reported techniques. First, it achieves faster analysis with a runtime of 10 minutes compared to 15-20 minutes in existing methods. Second, the method demonstrates superior sensitivity with LOQ values of 0.45 µg/mL for PTZ and 0.75 µg/mL for VAL, representing a 2-fold improvement over published methods. Third, the simple

mobile phase composition and gradient elution program make it more cost-effective and environmentally friendly compared to methods using complex buffer systems.

Table-5: Robustness Studies of Valsartan and Polythiazide

Parameters	Polythiazide (PT) RSD %	Valsartan (VAL) RSD %
Flow Rate	0.17 %	0.22 %
Wavelength	0.34 %	0.29 %
Column Oven Temperature	0.26 %	0.31 %

Fourth, the method's stability-indicating nature and comprehensive validation make it suitable for routine quality control analysis. Finally, the method's robustness and reproducibility, coupled with simple sample preparation steps, make it particularly suitable for high-throughput analysis in pharmaceutical quality control laboratories. These advances directly contribute to Sustainable Development Goal 3 (Good Health and Well-being) through improved pharmaceutical quality control ability for antihypertensive drugs. Hypertension is a major risk factor for cardiovascular disease worldwide, and the assurance of the quality of combination therapies such as VAL-PTZ plays a critical role in effective disease management. Through the supply of a valid, efficient analytical tool for this significant drug combination, this work increases medication safety and efficacy and thus contributes ultimately to improved health outcomes for hypertensive patients.

CONCLUSION

This research effectively established and validated a fast gradient RP-HPLC technique for the simultaneous quantification of Valsartan and Polythiazide in drug products. The technique provides considerable improvements over current methods, such as reduced analysis time (10-minute run), better sensitivity (LOQ: 0.45 µg/mL for PTZ, 0.75 µg/mL for VAL), and less complex mobile phase composition. The validated technique shows excellent linearity, precision, accuracy, and robustness, which qualify it for routine quality control use. By facilitating consistent quality determination of this valuable combination antihypertensive, this analytical strategy directly contributes to Sustainable Development Goal 3 (Good Health and Well-being) by improving medication safety and efficacy. This pharmaceutical quality control contribution ultimately contributes to improved blood pressure management, a key risk factor for cardiovascular disease, and thus addresses one of SDG 3's top goals: preventing premature mortality from non-communicable diseases.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well-being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID ids, given below:

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