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Research Article

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD FOR THE ESTIMATION OF A COMBINATION OF PRAVASTATIN SODIUM AND VALSARTAN BY REVERSED PHASE-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

Objective: The main aim of this study was to develop and validate analytical methods for an estimation of a combination of two different drugs by high performance liquid chromatography (HPLC). The objective of this study was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection, limit of quantification, and robustness.

Methods: Various parameters were analyzed for a drug combination according to standard procedures. The aim of this study was to develop a simple, accurate, and precise HPLC method for the analysis of the combination of pravastatin sodium (PVS) and valsartan using mobile phase and commonly employed Nucleodur C_{18} column with ultraviolet detector at 238 nm. The typical chromatogram of PVS and valsartan was shown in Fig. 5. The optimal retention time was found to be 4.815 minutes for PVS and 15.518 minutes for valsartan.

Result: The result of linearity for both PVS and valsartan was given in Tables 1 and 2, respectively. The results are shown in Tables 3 and 4. The repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the % relative standard deviation (RSD). The results of method precision are shown in Table 4. The % RSD for method precision was found to be 0.79 for PVS and 0.32 for valsartan. The results are shown in Tables 5 and 6. The ruggedness of analytical method is the degree of reproducibility of the test results obtained by the same samples under a variety of conditions such as different laboratories, different analysts, different instruments, different lots of reagents, and different days. The assay result indicated that the method was capable with a high precision. The results of % RSD prove the ruggedness of developed method as shown in Tables 7 and 8.

Conclusion: The proposed reversed phase-HPLC method enables the determination of PVS and valsartan because of a good separation of chromatographic peaks (Fig. 5). The method can be used successfully for the analysis of PVS and valsartan in combination.

Keywords: Pravastatin sodium, Valsartan, High performance liquid chromatography, Validation.

INTRODUCTION

Pravastatin sodium (PVS) is a member of the 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors class of lipid-lowering drugs. PVS is a selective, competitive inhibitor of HMG-CoA reductase, the rate-limiting enzyme that converts 3-hydroxy-3 methyl glutaryl-coenzyme A to mevalonate, a precursor of the sterols including cholesterol. It is mainly used for the treatment of hypercholesterolemia and prevention of cardiovascular disease. PVS is chemically, Sodium(3*R*,5*R*)-3,5-dihydroxy-7-[(1*S*,2*S*,6*S*,8*S*,8a*R*)-6-hydroxy-2-methyl-8-[[(2*S*)-2-methylbutanoyl]oxy]-1, 2, 6, 7, 8, 8 ahexahydronaphthalen-1-yl] heptanoate [1,2].

Valsartan is chemically 3-methyl-2- [pentanoyl-[[4-[2-(2H-tetrazol-5-yl) phenyl] phenyl] methyl]amino] -butanoic acid (Fig. 2), angiotensin II receptor antagonist, acting on the AT1 subtype and used for the treatment of high blood pressure, congestive heart failure, and post-myocardial infarction. By blocking the action of angiotensin, valsartan dilates blood vessels and reduces blood pressure [3,4].

A survey of literature has revealed that there are a very few high performance liquid chromatography (HPLC) methods available for the determination of a combination of PVS and valsartan. However, HPLC studies have been performed individually on both the drugs. The main objective was to develop and validate HPLC method for the combination of PVS and valsartan, as few research studies indicate that statins and angiotensin II Type 1 receptor blocker therapy improves endothelial dysfunction using distinct mechanisms. Evaluations were made on simultaneous vascular and metabolic responses to PVS and valsartan therapy, alone or in combination, in hypercholesterolemic patients. There was simultaneous improvement in metabolic phenotypes, with all three treatments causing increased plasma adiponectin levels, reduced fasting insulin levels, and increased insulin sensitivity relative to baseline measurements. The studies show that in a statin combination trial, pravastatin combined with valsartan therapy increased plasma adiponectin, lowered fasting insulin levels, and improved insulin sensitivity in an additive manner when compared with monotherapy alone [5].

METHODS

Instrumentation

The HPLC (Shimadzu, Kyoto, Japan) instrument was equipped with two LC-10 ATVP pumps, SPD-10AVP ultraviolet (UV)-visible detector, and Rheodyne injector with 50 µl loop. The results were acquired and processed using Shimadzu LC-solution version 6.42 software for data acquisition and processing.

Column: Nucleodur C18 (250 mm × 4.6 mm I.D., 5 µm).

Chemicals and reagents

HPLC grade methanol, glacial acetic acid, acetonitrile, and triethylamine were procured from Thermo Fischer scientific India Pvt. Ltd., and potassium dihydrogen phosphate was purchased from Merck

specialties Pvt. Ltd., Mumbai. The active pharmaceutical ingredients PVS and valsartan were obtained from Oniosome Research Centre.

Chromatographic condition
The mobile phase was composed of acetonitrile:water:glacial acetic acid in the ratio of 450:550:0.1, on a reversed phase (RP) C_{18} column. The flow rate was 1.0 ml/minutes and the detection wavelength was 238 nm. The injected volume was 20 µL.

Preparation of the mobile phase and diluent
The mobile phase was composed of buffer (0.025 of glacial acetic acid in 250 ml of water) and acetonitrile. The solution was filtered through 0.45 µm filter under vacuum and degassed in an ultrasonic water bath for 10 minutes. A mixture of methanol:water (50:50) was used as diluent.

Standard preparation
A stock solution of PVS and valsartan was prepared by accurately weighing 10 mg drug, transferring to 1 ml appendroff, dissolving in 1 ml of a diluent methanol:water (50:50) and was sonicated to dissolve. Then, further dilutions were made to obtain the final standard solution of 100 µg/ml.

Preparation of sample solution
From the 1 µg/ml dilutions each of PVS and valsartan, 0.1 ml was taken into an appendroff and volume was made up to 1 ml with diluent. The sample solution was filtered through 0.25 µm filter.

Method validation
The objective of the method validation is to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision, accuracy, specificity, and limit of detection (LOD), limit of quantification (LOQ), and robustness.

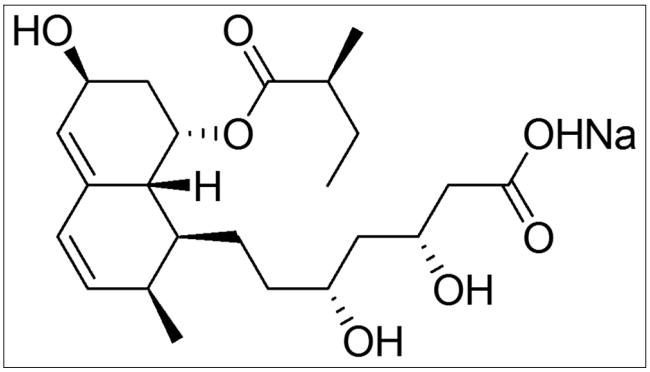

Fig. 1: Chemical structure of pravastatin sodium

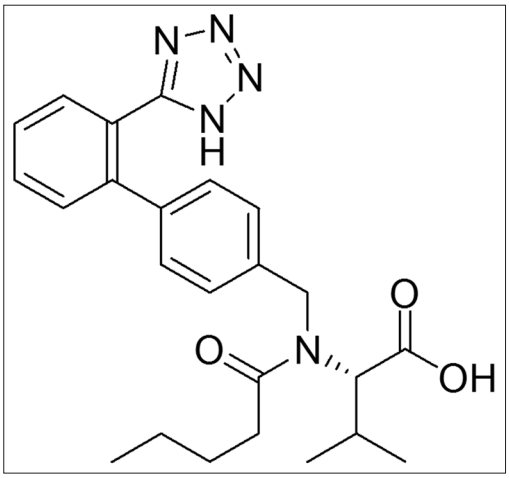

Fig. 2: Chemical structure of valsartan

Linearity
To evaluate the linearity of PVS, serial dilution of analyte was prepared from the stock solution by taking suitable volume and diluted up to 1 ml to get the desired concentrations (0.125, 0.250, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 µg/ml) for linearity in the range of 0.125-10 µg/ml. Likewise, the dilutions for valsartan were made to get the desired concentrations (1.25, 2.5, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 µg/ml) for linearity in the range of 1.25-100 µg/ml. The prepared solutions were filtered through 0.45 µm membrane filter and each of the dilutions was injected three times into the column. Absorbance at 238 nm was measured, and a calibration curve for PVS and valsartan was constructed by plotting the mean peak area (Y-axis) against the concentration (X-axis).

Precision
The precision of the method was investigated with respect to repeatability and ruggedness.

Interday and intraday
Intraday precision was calculated from six replicate injections of freshly prepared PVS and valsartan test solution in the same equipment at a concentration value of 5 and 50 µg/ml, respectively, on the same day after regular interval. The interday experiment was repeated by assaying freshly prepared solution at the same concentration, in addition, on 2 consecutive days to determine interday intermediate precision.

Method precision (repeatability)
The method precision for assay was established by determining the assay of six sample preparations under the same conditions. Six replicates of PVS (5 µg/ml) and valsartan (50 µg/ml) sample solution were prepared at sample concentration by one analyst and analyzed on the same day.

Intermediate precision (ruggedness)
Different analyst, using a different system, repeated the procedure followed for method precision on a different day using same lot of sample.

Accuracy
The accuracy was determined over the range 80-120% of the sample concentration. Calculated amount of PVS and valsartan from standard stock solution was added in placebo to attain 80%, 100% and 120% of the sample concentration. Each sample was prepared in triplicate at each level. Blank and standard preparations were injected, and the chromatograms were recorded.

Specificity
Specificity is a procedure to detect quantitatively the analyte in the presence of component that may be expected to be present in the sample matrix. Commonly used excipients in tablet preparation of PVS and Valsartan were spiked in a pre-weighed the quantity of drugs, and then, the absorbance was measured and calculations done to determine the quantity of the drugs.

Robustness
The method was found to be robust, as small but deliberate changes in the method parameters have no detrimental effect on the method performance. Change in flow rate of mobile phase to 1.19 ml/minutes and 1.21 ml/minutes (±10%) or column oven temperature (±5°C absolute) to 35°C and 45°C or organic phase ratio of mobile phase by (±2% absolute) as acetonitrile:water and glacial acetic acid buffer (43:57), acetonitrile:water and glacial acetic acid buffer (47:53) or change in wavelength (±5 nm) to 233 nm and 243 nm and to observe their effect on system suitability.

Detection and quantification limit
LOD and LOQ were calculated by the proposed method which was based on the standard deviation (s) of the response and the slope (S) of the calibration curve at levels approximating the LOD and LOQ,

LOD = 3.3 (σ/S) and LOQ = 10 (σ/S).

RESULT AND DISCUSSION

Method development

The aim of this study was to develop a simple, accurate, and precise HPLC method for the analysis of the combination of PVS and valsartan using mobile phase and commonly employed Nucleodur C_{18} column with UV detector at 238 nm. The typical chromatogram of PVS and valsartan was shown in Fig. 5. The optimal retention time was found to be 4.815 minutes for PVS and 15.518 minutes for valsartan.

Method validation

The described method has been validated for the assay of drugs combination as an active pharmaceutical ingredient. The method was validated for the parameters such as specificity, linearity, LOD, LOQ, accuracy, precision, ruggedness, and robustness [6,7].

Linearity

The range of an analytical method is the interval between the upper and lower analytical concentration of a sample where the method has shown to demonstrate acceptable accuracy, precision, and linearity [8]. The linearity was studied by preparing standard solutions at different concentration levels. The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of an analyte in samples within a given range. The linearity of the method was observed in the expected concentration range that demonstrated its suitability for analysis. The calibration curve was carried out and found to be linear in the concentration range of 0.125-10 µg/ml for PVS (Fig. 3) and 1.25-100 µg/ml for valsartan (Fig. 4). The result of linearity for both PVS and valsartan was given in Tables 1 and 2, respectively.

Precision

Precision is a measure of the ability of the method to generate reproducible results. The precision of the method was evaluated using interday, intraday, repeatability, and ruggedness. The % relative standard deviation (RSD) in the case of PVS for interday was found to be 1.405 and intraday was 0.97, whereas for valsartan interday % RSD was 0.43 and intraday was 0.50. The results are shown in Tables 3 and 4. Repeatability refers to the use of the analytical procedure within a laboratory over a short period of time using the same analyst with the same equipment and is expressed as the % RSD. The results of method precision are shown in Table 4. The % RSD for the method precision was found to be 0.79 for PVS and 0.32 for valsartan. The results are shown in Tables 5 and 6. The ruggedness of an analytical method is the degree of reproducibility of the test results obtained by the same samples under a variety of conditions such as different laboratories, different analysts, different instruments, different lots of reagents, and different days. The assay result indicated that the method was capable with a high precision. The results of % RSD prove the ruggedness of developed method as shown in Tables 7 and 8.

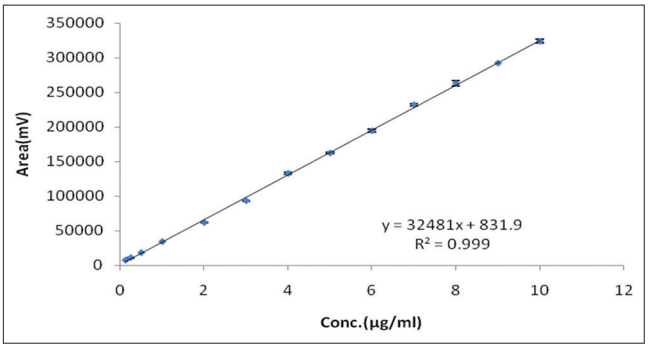

Fig. 3: Linear calibration curve for pravastatin sodium

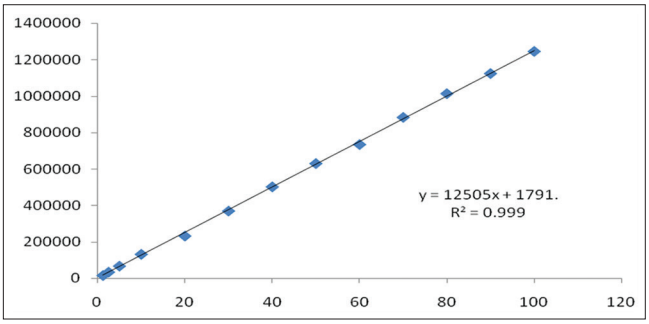

Fig. 4: Linear calibration curve for valsartan

Table 1: Data derived from linearity experiment of PVS

S. No.	Concentrated (µg/ml)	Mean±SD	% RSD
1.	0.125	7077.667±11.93035	0.168563
2.	0.250	10709±158.6064	1.481057
3.	0.5	17896.33±14.57166	0.081423
4.	1	33853.33±47.85743	0.141367
5.	2	61570±668.7159	1.086107
6.	3	93135±738.4822	0.792916
7.	4	132780.3±1819.841	1.370565
8.	5	162300±1358.528	0.837047
9.	6	194522.7±2081.849	1.070235
10.	7	232106.3±1785.874	0.769421
11.	8	262974±3571.254	1.358026
12.	9	292538±164.5387	0.056245
13.	10	324228±3030.06	0.934546

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 2: Data derived from linearity experiment of valsartan

S. No.	Concentrated (µg/ml)	Mean±SD	% RSD
1.	1.25	18017±15.6205	0.086699
2.	2.5	36251±143.6802	0.396348
3.	5	69384.33±993.974	1.432563
4.	10	134219.7±868.7234	0.64724
5.	20	234378.7±428.5048	0.182826
6.	30	371507.7±368.9232	0.099304
7.	40	504190.3±247.114	0.049012
8.	50	632237.3±1580.456	0.249978
9.	60	736416±4625.763	0.628145
10.	70	885405.3±1254.873	0.141729
11.	80	1015402±4596.483	0.452676
12.	90	1125772±4260.183	0.378423
13.	100	1247170±2705.713	0.216948

SD: Standard deviation, RSD: Relative standard deviation

Table 3: Data derived from precision experiment for PVS

S. No.	Concentrated (µg/ml)	Peak area	
		(interday)	(intraday)
1.	5	163,139	165,838
2.	5	165,974	163,716
3.	5	164,576	165,758
4.	5	160,391	167,147
5.	5	160,157	163,375
6.	5	163,166	163,299
7.	SD	2289.343	1608.963
8.	Mean	162900.5	164855.5
9.	% RSD	1.405363	0.975984

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Accuracy

The accuracy of an analytical method is the closeness in the agreement between the accepted true value or a reference value and the actual result obtained. Accuracy studies are usually evaluated by determining the recovery of a spiked sample of the analyte into the matrix of the sample to be analyzed [9]. The results of accuracy studies are shown in Tables 9 and 10. Results of recoveries and coefficient of variation (% RSD) indicate that the method is accurate within the desired range.

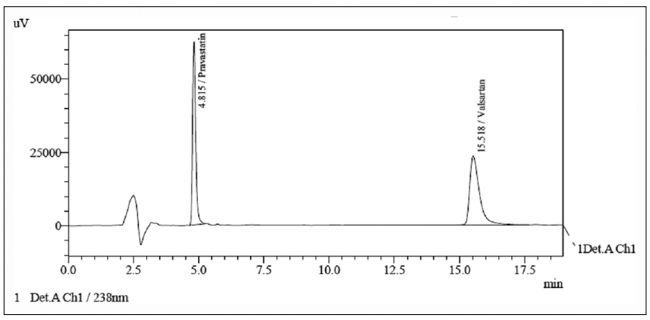

Fig. 5: Chromatogram of a combination of pravastatin sodium and valsartan

Table 4: Data derived from precision experiment for valsartan

S. No.	Concentrated (µg/ml)	Peak area	
		(interday)	(intraday)
1.	50	631,053	636,047
2.	50	634,756	634,604
3.	50	634,434	632,619
4.	50	632,665	637,150
5.	50	638,077	639,853
6.	50	637,691	630,963
7.	SD	2750.57	3198.095
8.	Mean	634779.3	635206
9.	% RSD	0.433311	0.503474

SD: Standard deviation, RSD: Relative standard deviation

Table 5: Data derived from repeatability experiment for PVS

S. No.	Concentrated (µg/ml)	Area	X	Assay
1.	5	163,357	4.991767	100.1649
2.	5	162,165	4.955228	100.9035
3.	5	162,048	4.951641	100.9766
4.	5	164,402	5.023799	99.52627
5.	5	161,088	4.922214	101.5803
6.	5	164,048	5.012948	99.74171
7.	SD	1289.808		
8.	Mean	162851.3		
9.	% RSD	0.792015		

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 6: Data derived from repeatability experiment for valsartan

S. No.	Concentrated (µg/ml)	Area	X	Assay
1.	50	635,362	50.63269	98.75043
2.	50	638,559	50.88778	98.25542
3.	50	632,995	50.44383	99.12015
4.	50	638,195	50.85873	98.31153
5.	50	637,522	50.80503	98.41544
6.	50	636,375	50.71352	98.59305
7.	SD	2085.794		
8.	Mean	636501.3		
9.	% RSD	0.327697		

SD: Standard deviation, RSD: Relative standard deviation

Specificity

Specificity is the ability of a method to discriminate between the analyte of interest and other components that are present in the sample [9,10]. The method demonstrated good separation between the peaks and

Table 7: Data derived from intermediate precision for PVS

S. No.	Concentrated (µg/ml)	Area
	Analyst 1	
1.	5	178,021
2.	5	179,300
3.	5	177,785
4.	SD	815.1444
5.	Mean	178368.7
6.	% RSD	0.457
	Analyst 2	
1.	5	175,273
2.	5	172,584
3.	5	173,806
4.	SD	1346.359
5.	Mean	173887.7
6.	% RSD	0.774269

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 8: Data derived from intermediate precision for valsartan

S. No.	Concentrated (µg/ml)	Area
	Analyst 1	
1.	50	1,738,296
2.	50	1,738,330
3.	50	1,739,840
4.	SD	881.7777
5.	Mean	1,738,822
6.	% RSD	0.050711
	Analyst 2	
1.	50	1,750,612
2.	50	1,750,610
3.	50	1,738,290
4.	SD	7113.533
5.	Mean	1,746,504
6.	% RSD	0.407301

SD: Standard deviation, RSD: Relative standard deviation

Table 9: Data derived from accuracy experiment for PVS

S. No.	80%	
	Concentrated (µg/ml)	Area
1.	4	132425
2.	4	132143
3.	4	134513
4.	SD	1294.615
5.	Mean	133027
6.	%RSD	0.973197
	100%	
1.	5	159,317
2.	5	156,204
3.	5	153,701
4.	SD	2813.516
5.	Mean	156,407.3
6.	%RSD	1.798839
	120%	
1.	6	183,630
2.	6	180,586
3.	6	185,035
4.	SD	2274.26
5.	Mean	183,083.7
6.	%RSD	1.242197

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

was found to be free of interference. For demonstrating the specificity of the method for drug formulation, the drug was spiked, wherein the excipients used in different formulation products did not interfere with the drug peak and thus the method was specific for the combination of PVS and valsartan. The results are shown in Tables 11 and 12.

Table 10: Data derived from accuracy experiment for valsartan

S. No.	80%	
	Concentrated (µg/ml)	Area
1.	40	497234
2.	40	492223
3.	40	495634
4.	SD	2559.461
5.	Mean	495030.3
6.	%RSD	0.517031
	100%	
1.	50	645662
2.	50	642732
3.	50	642020
4.	SD	1930.285
5.	Mean	643471.3
6.	% RSD	0.29998
	120%	
1.	60	735894
2.	60	733740
3.	60	733057
4.	SD	1480.697
5.	Mean	734230.3
6.	% RSD	0.201666

SD: Standard deviation, RSD: Relative standard deviation

Table 11: Data derived from specificity experiment for PVS

S. No.	Concentrated (µg/ml)	Area
1.	5	168,727
2.	5	165,618
3.	5	168,948
4.	SD	1862.061
5.	Mean	167764.3
6.	% RSD	1.109926

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 12: Data derived from specificity experiment for valsartan

S. No.	Concentrated (µg/ml)	Area
1.	50	1,889,617
2.	50	1,856,988
3.	50	1,888,181
4.	SD	18,437.81
5.	Mean	1,878,262
6.	% RSD	0.981642

SD: Standard deviation, RSD: Relative standard deviation

Table 13A: Data derived from robustness experiment for PVS: Change in column temperature

S. No.	Concentrated (µg/ml)	Temperature		
		35°C	40°C	45°C
1.	5	134,143	160,973	134,454
2.	5	130,635	163,688	130,920
3.	5	133,640	162,239	134,554
4.	SD	1896.888	1358.528	2069.827
5.	Mean	132806	162300	133309.3
6.	% RSD	1.428315	0.837047	1.55265

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Robustness

This was done by small deliberate changes in the chromatographic conditions. The results of robustness study are summarized in Tables 13 and 14, indicating that the method was robust enough that the selected factors remained unaffected by small variations of these parameters.

Detection and quantification limit

The detection limit or LOD is the lowest amount of analyte in a sample that can be detected, but not necessarily quantitated. LOD was expressed as a concentration that gives a signal to noise ratio of 2:1 or 3:1. Quantitation limit or LOQ, on the other hand is the lowest amount of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. LOQ is measured in terms of signal to noise ratio of 10:1. LOD and LOQ

Table 13B: Data derived from robustness experiment for PVS: Change in wavelength

S. No.	Concentrated (µg/ml)	233 nm	238 nm	243 nm
1.	5	152,983	160,973	143,349
2.	5	151,616	163,688	145,504
3.	5	151,341	162,239	143,715
4.	SD	879.439	1358.528	1153.148
5.	Mean	151,980	162,300	144189.3
6.	% RSD	0.578654	0.837047	0.799746

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 13C: Data derived from robustness experiment for PVS: Change in flow rate

S. No.	Concentrated (µg/ml)	ml/minutes		
		1.19	1.2	1.21
1.	5	135,139	160,973	135,279
2.	5	138,433	163,688	135,146
3.	5	135,734	162,239	133,418
4.	SD	1755.423	1358.528	1038.187
5.	Mean	136,435.3	162,300	134,614.3
6.	% RSD	1.286634	0.837047	0.771231

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 13D: Data derived from robustness experiment for PVS: Change in mobile phase

S. No.	Concentrated (µg/ml)	43:57	45:55	47:53
1.	5	113,637	160,973	112,883
2.	5	113,229	163,688	110,866
3.	5	111,800	162,239	111,045
4.	SD	964.6307	1358.528	1116.436
5.	Mean	112888.7	162300	111598
6.	% RSD	0.854497	0.837047	1.000408

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation

Table 14A: Data derived from robustness experiment for valsartan: Change in column temperature

S. No.	Concentrated (µg/ml)	Temperature		
		35°C	40°C	45°C
1.	50	641,075	633,691	648,139
2.	50	644,463	630,555	643,485
3.	50	642,519	639,466	640,407
4.	SD	1700.138	4520.159	3892.677
5.	Mean	642,685.7	634,570.7	644,010.3
6.	% RSD	0.264536	0.712318	0.604443

SD: Standard deviation, RSD: Relative standard deviation

Table 14B: Data derived from robustness experiment for valsartan: Change in wavelength

S. No.	Concentrated (µg/ml)	233 nm	238 nm	243 nm
1.	50	766,465	633,691	534,527
2.	50	764,730	630,555	537,601
3.	50	768,453	639,466	536,359
4.	SD	1862.932	4520.159	1546.408
5.	Mean	766549.3	634570.7	536162.3
6.	% RSD	0.243028	0.712318	0.288422

SD: Standard deviation, RSD: Relative standard deviation

Table 14C: Data derived from robustness experiment for valsartan: Change in flow rate

S. No.	Concentrated (µg/ml)	ml/minutes		
		1.19	1.2	1.21
1.	50	649,466	633,691	647,499
2.	50	644,615	630,555	643,960
3.	50	644,755	639,466	642,253
4.	SD	2761.199	4520.159	2675.783
5.	Mean	646278.7	634570.7	644570.7
6.	% RSD	0.427246	0.712318	0.415126

SD: Standard deviation, RSD: Relative standard deviation

Table 14D: Data derived from robustness experiment for valsartan: Change in mobile phase

S. No.	Concentrated (µg/ml)	43:57	45:55	47:53
1.	50	642,527	633,691	648,822
2.	50	641,453	630,555	646,365
3.	50	647,809	639,466	646,909
4.	SD	3402.248	4520.159	1290.501
5.	Mean	643,929.7	634,570.7	647,365.3
6.	% RSD	0.528357	0.712318	0.199347

SD: Standard deviation, RSD: Relative standard deviation

Table 15: Characteristics of the analytical method derived from the standard calibration curve

S. No.	Compound	Linearity (µg/ml)	Correlation co-efficient (R^2)	Residual standard regression (σ)	Slope of regression (S)	LOD (µg/ml)	LOQ (µg/ml)
1.	PVS	0.125-10	0.999	462.0768	32481	0.046946	0.142261
2	Valsartan	1.25-100	0.999	308.7572	12505	0.081479	0.246907

PVS: Pravastatin sodium, SD: Standard deviation, RSD: Relative standard deviation, LOD: Limit of detection, LOQ: Limit of quantification

were calculated by the equation given in ICH guidelines. This may be expressed as LOD = 3.3 σ/S and LOQ = 10 σ/S, where σ is the standard deviation of the response, S is the slope of the calibration curve which may be estimated from the calibration curve of the analyte [9,10]. The LOD and LOQ of the proposed method at 238 nm, for PVS were found to be 0.04 and 0.14 µg/ml, respectively, shown in Table 15, whereas 0.08 and 0.24 for valsartan. The proposed high performance liquid chromatographic method has been evaluated as per ICH guidelines, parameters such as linearity, precision, accuracy, LOD, LOQ, specificity, and robustness are proved to be convenient for the quality control of PVS and valsartan in combination form [11]. The proposed RP-HPLC method enables the determination of PVS and valsartan because of good separation of chromatographic peaks (Fig. 5). The method can be used successfully for the analysis of PVS and valsartan in combination.

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