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Research Article

Development and Validation RP-HPLC Method for Simultaneous Estimation of Valsartan and Nifedipine in Synthetic Mixture

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ABSTRACT

A simple, specific and accurate Reverse Phase High Performance Liquid Chromatography Method was developed for the simultaneous determination of Valsartan and Nifedipine in Synthetic Mixture. The using Phenomenex Luna C_{18} (250 mm x 4.6 mm, 5 µm) column in Isocratic mode, with Mobile Phase containing Methanol: Water: ACN (pH 3.7 adjusted with 10% Ortho Phosphoric Acid) (70:25:05 %v/v/v). The Flow Rate was 1 ml/min and effluents were monitored at 233 nm. The Retention Time of Valsartan and Nifedipine were found to be 8.003 min and 5.290 min respectively. The Linearity for Valsartan and Nifedipine were found to be 4-20 µg/ml and 1.5-7.5 µg/ml respectively. The Recoveries of Valsartan and Nifedipine were found to be 99.50% – 100.15% and 99.33% – 100.40% respectively. The proposed method was validated and successfully applied for the estimation of Valsartan and Nifedipine in Synthetic Mixture.

Key-words: Valsartan, Nifedipine, RP-HPLC.

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INTRODUCTION:[1]

Valsartan is an Angiotensin II Receptor Antagonist (commonly called an ARB, or angiotensin receptor blocker), that is selective for the type I (AT1) angiotensin receptor. It is used to treat high blood pressure, congestive heart failure, and to reduce death for people with left ventricular dysfunction after having had a heart attack.

Nifedipine is a dihydropyridine calcium channel blocker that primarily blocks L-type calcium channels. Its main uses are as an antianginal and antihypertensive, although a large number of other indications have recently been found for this agent, such as Raynaud's phenomenon, premature labour, and painful spasms of the esophagus such as in cancer and tetanus patients.

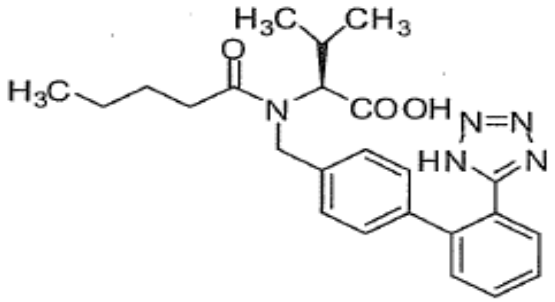

Figure 1: Valsartan

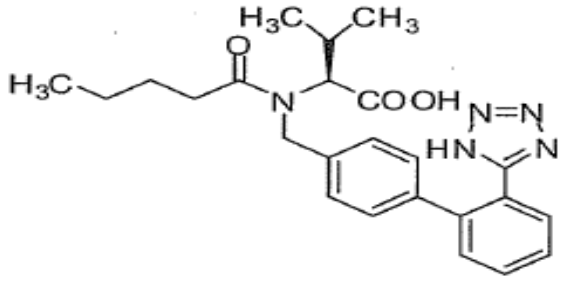

Figure 2: Nifedipine

INSTRUMENTAL:

- HPLC (Shimadzu)
  Model: SPD-20A, LC-20AD
  Column: Phenomenex Luna C_{18} (250 mm x 4.6 mm, 5 µm)
  Detector: U.V Detector
  Software: Spinchrome
- Hamilton Syringe
- Analytical Weighing Balance (Wensar DAB-220)
- Sonicator (Equitron)
- Digital pH Meter (Systronic)
- High Vacuum Pump (Parag engineering)

CHEMICALS AND MATERIALS:

- Acetonitrile – Avantor Performance Material India Ltd. (HPLC grade)
- Methanol – Finar Ahmedabad. (HPLC grade)
- Water – Astron Chemical India. (HPLC grade)
- OPA (75% Ortho Phosphoric Acid) - AR Grade, Astron Chemical India.
- Valsartan and Nifedipine were supplied by Torrent Pharmaceuticals, Ahmedabad, India and Mediwin Pharmaceutical, Ahmedabad, India respectively.

SELECTION OF DETECTION WAVELENGTH:

- The sensitivity of HPLC method that uses UV detection depends upon proper selection of detection wavelength. At 233 nm both the drug give good peak height and shape. So, 233 nm was selected for detection of Valsartan and Nifedipine in RP-HPLC system.
  (Figure 3)

MOBILE PHASE SELECTION:

The composition and flow rate of mobile phase were changed to optimize the separation condition using combined solution. The pKa value for Valsartan and Nifedipine is 4.37 and 5.33 respectively. After number of trial experiments, it was established that the mobile phase Methanol: Water: ACN (pH 3.7 adjusted with 10% Ortho Phosphoric Acid) (70:25:05 %v/v/v) shows good peak shape and resolution.

CHROMATOGRAPHIC CONDITION:

- **Column:** Phenomenex LunaC_{18} (240 mm × 4.6 mm, 5 µm)
- **Mobile Phase:** Methanol: Water: ACN (pH 3.7 adjusted with 10% Ortho Phosphoric Acid) (70:25:05 %v/v/v)
- **Flow Rate:** 1 ml/min
- **Detection Wavelength:** 233 nm
- **Run Time:** 15 min
- **Detector:** U.V Detector
- **Injection Volume:** 20 µL

PREPARATION OF STANDARD STOCK SOLUTION:

- **Valsartan (100 µg/ml):**

Accurately weighed VAL (10 mg) was transferred to a 100 ml volumetric flask, and diluted to the mark with Methanol to obtain a standard stock solution (100 µg/ml).

- **Nifedipine (100 µg/ml):**

Accurately weighed NIFE (10 mg) was transferred to a 100 ml volumetric flask, and diluted to the mark with Methanol to obtain a standard stock solution (100 µg/ml).

PREPARATION AND ANALYSIS OF SYNTHETIC MIXTURE [2]:

- The Synthetic Mixture of Valsartan and Nifedipine was prepared in ratio of 80:30.
- Common excipients, Lactose (95 mg), MCC [Micro Crystalline Cellulose] (129 mg), Magnesium Stearate (9 mg), Talc (7 mg) along with the drug Valsartan 80 mg and Nifedipine 30 mg.
- Accurately weighed equivalently weight of Valsartan 80 mg which contain Valsartan (80 mg) and Nifedipine (30 mg) which transferred in 100 ml volumetric flask and make up half mark with Methanol. This solution was sonicated till the drug dissolves and was made upto mark with Methanol. Then this solution was filtered through Whatmann filter paper. So, obtained concentration of Valsartan is 800 µg/ml and Nifedipine is 300 µg/ml.

PREPARATION OF WORKING SAMPLE SOLUTION:

From above synthetic mixture solutions take 0.1 ml and transferred in to a 10 ml volumetric flask and the volume was adjusted up to the mark with Mobile Phase to make final concentration of VALSARTAN 8 µg/ml and NIFEDIPINE 3 µg/ml. (Table 6)

METHOD VALIDATION [3]:

The developed method was validated with respect to specificity, linearity, range, accuracy, and precision, limit of detection and limit of quantification in accordance with the ICH guideline.

➢ **Specificity:**

Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.

➢ **Linearity & Range:**

The linearity of VALSARTAN and NIFEDIPINE was found to be in the range of 4-20 µg/ml and 1.5-7.5 µg/ml respectively. Plot the calibration curve of Area (µV.s) vs. Concentration (µg/ml). Linearity of both the drugs was checked in term of Slope, Intercept and Correlation Coefficient. (Table 1)

➢ **Precision:**

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. Precision may be considered at three levels: Intermediate (Intraday) Precision, Reproducibility (Interday) Precision, and Repeatability.(Table 2, 3)

1) Intraday Precision:

Solutions containing 8, 12, 16 µg/ml of VAL and 3, 4.5, 6 µg/ml of NIFE were analyzed three times on the same day and %R.S.D was calculated.

2) Interday Precision:

Solutions containing 8, 12, 16 µg/ml of VAL and 3, 4.5, 6 µg/ml of NIFE were analyzed on three different successive days and %R.S.D was calculated.

3) Repeatability:

Solutions containing 12 µg/ml of VAL and 4.5 µg/ml of NIFE were analyzed for six times and %R.S.D. was calculated.

➢ **Accuracy:**

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Recovery studies were carried out by addition of standard drug to the sample at three different concentration levels 50%, 100%, 150%. This performance was done in triplicate. The amount of VALSARTAN and NIFEDIPINE were calculated at each level and % recoveries were calculated by measuring the Peak Area and fitting the values in equation. (Table 4)

➢ **Limit of Detection (LOD):**

Limit of detection can be calculated using following equation as per ICH guidelines. (Table 5)

LOD = 3.3 x (σ/ S)

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

➢ **Limit of Quantification (LOQ):**

Limit of quantification can be calculated using following equation as per ICH guidelines. (Table 5)

LOQ = 10 x (σ/ S)

Where, σ = standard deviation of the Y intercept of calibration curve

S = Mean slope of the corresponding calibration curve.

➢ **Robustness:**

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. (Table 7)

It should show the reliability of an analysis with respect to deliberate variation in method parameter.

In case of liquid chromatography, examples of typical variations are:

- Influence of variations of pH in mobile phase;
- Influence of variations in mobile phase composition;
- Different columns (different lots and/or suppliers)
- Temperature
- Flow rate
-

➢ **System Suitability Tests:**

A system suitability test is an integral part of liquid chromatography. They are used to verify that resolution and reproducibility of chromatography system are adequate for the analysis to be done. The Test includes the Resolution, Column Efficiency, Tailing Factor and Theoretical Plates.

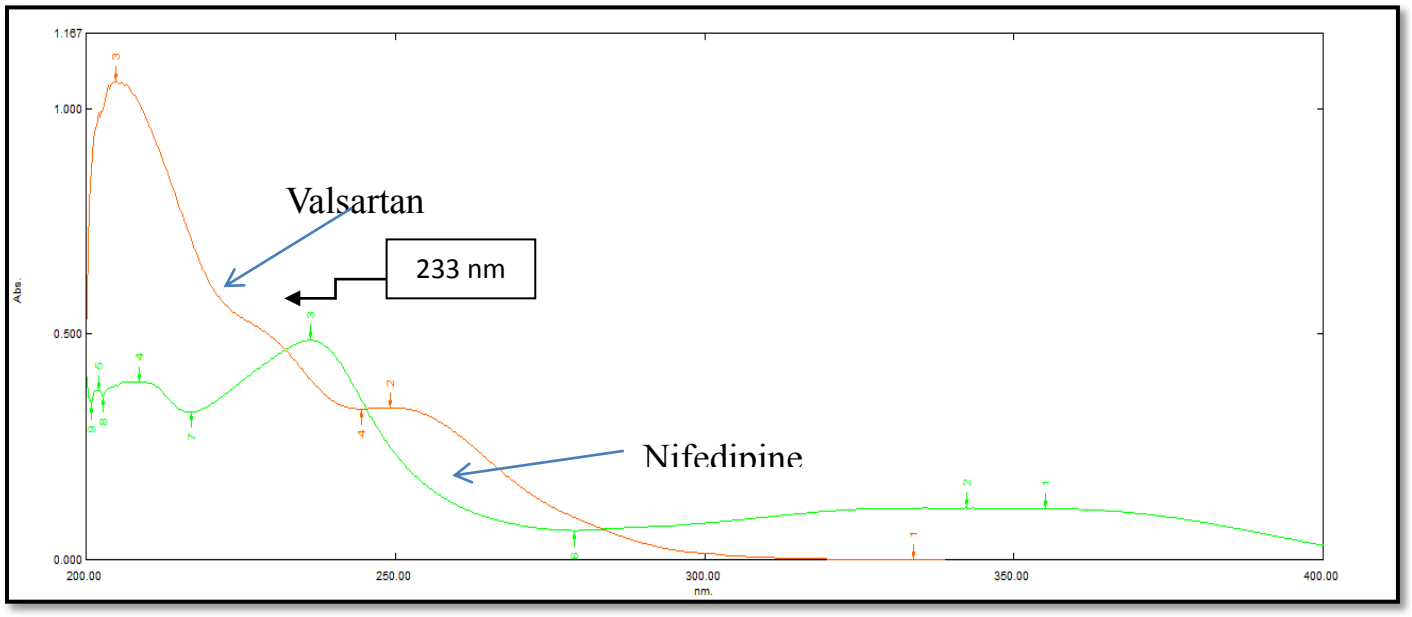

Figure 3: Selection of Wavelength (Overlain Spectra of Valsartan (10 µg/ml) and Nifedipine (8 µg/ml))

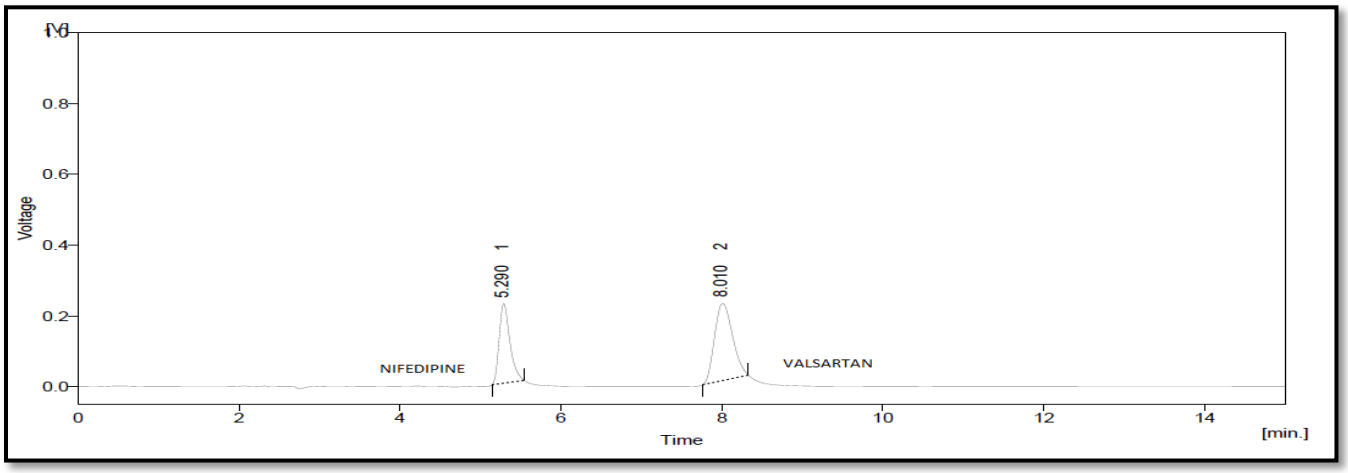

Figure 4: Chromatogram of VAL (8 µg/ml) and NIFE (3 µg/ml) in Methanol: Water: ACN (pH 3.7) (70:25:05 %v/v/v)

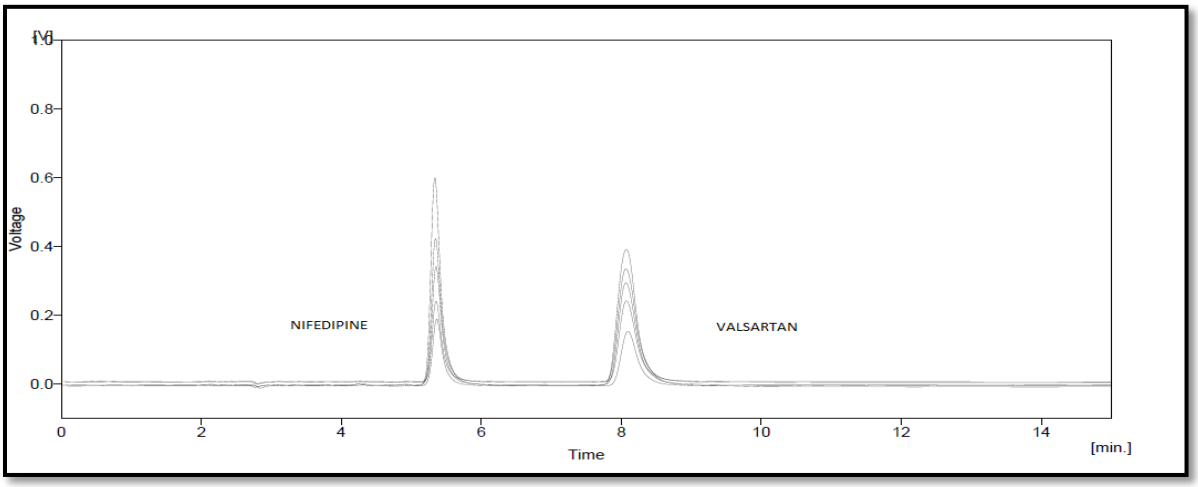

Figure 5: Overlay Chromatogram of Nifedipine (1.5-7.5 µg/ml) and Valsartan (4-20 µg/ml)

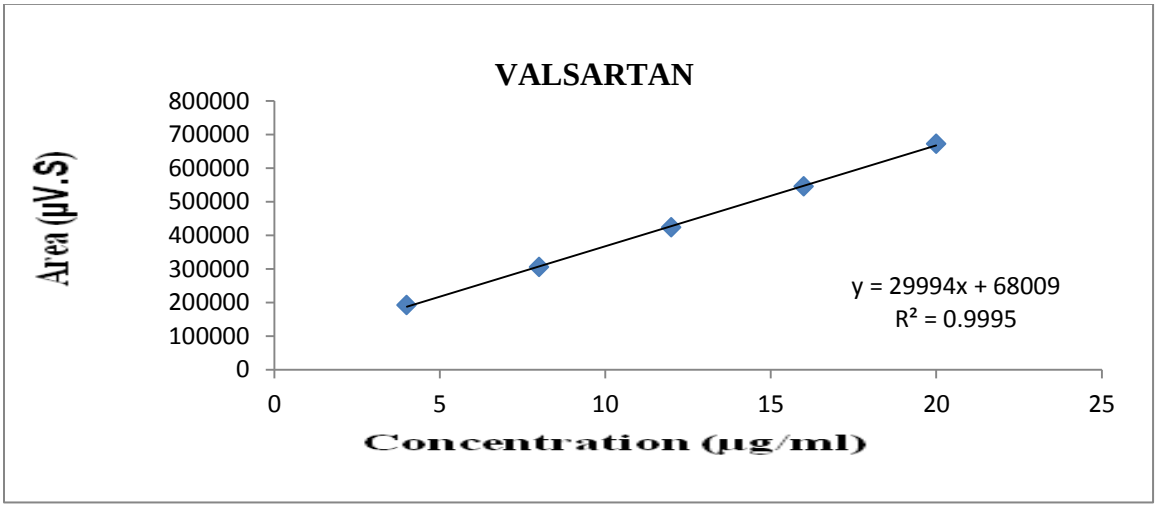

Figure 6: Calibration Curve of VALSARTAN (4-20 µg/ml)

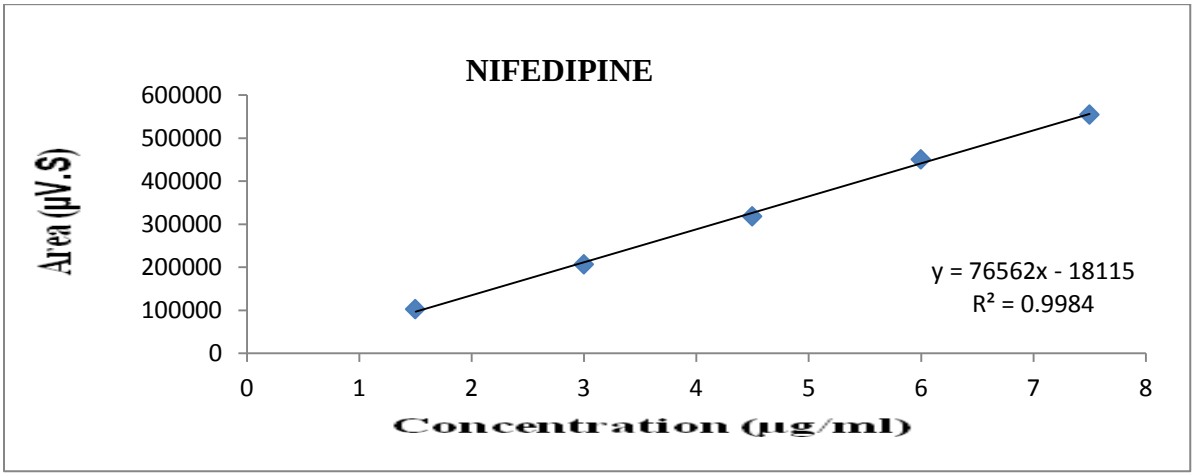

Figure 7: Calibration Curve of NIFEDIPINE (1.5-7.5 µg/ml)

Table 1: Linearity Data for VAL (4-20 µg/ml) and NIFE (1.5-7.5 µg/ml)

VALSARTAN			NIFEDIPINE		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD	Conc. (µg/ml)	Mean Peak Area (µV.s) ± S.D. (n=6)	% RSD
4	192308 ± 1294.68	0.6732	1.5	102180 ± 691.87	0.6771
8	305672 ± 1588.84	0.5197	3	206646 ± 1031.32	0.4990
12	424056 ± 1679.95	0.3961	4.5	318262 ± 1189.62	0.3737
16	545254 ± 1860.66	0.3412	6	450535 ± 1388.88	0.3082
20	672398 ± 1930.99	0.2871	7.5	554453 ± 1636.37	0.2951

Table 2: Precision Study for VALSARTAN

VALSARTAN		
INTRADAY PRECISION		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S. D. (n=3)	% R.S.D
8	308157 ± 1551.18	0.5033
12	424210 ± 1571.61	0.3704
16	546753 ± 1959.70	0.3584
INTERDAY PRECISION		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S. D. (n=3)	% R.S.D
8	307065 ± 1847.81	0.6017
12	427302 ± 2180.14	0.5102
16	548962 ± 2313.50	0.4214
REPEATABILITY		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S. D. (n=6)	% R.S.D
12	425756 ± 2399.31	0.5635

Table 3: Precision Study for NIFEDIPINE

NIFEDIPINE		
INTRADAY PRECISION		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S. D. (n=3)	% R.S.D
3	208491 ± 1001.66	0.4804
4.5	318766 ± 1213.05	0.3805
6	452956 ± 1351.31	0.2983
INTERDAY PRECISION		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S. D. (n=3)	% R.S.D
3	210878 ± 1158.16	0.5492
4.5	321398 ± 1543.43	0.4802
6	455000 ± 1992.11	0.4378
REPEATABILITY		
Conc. (µg/ml)	Mean Peak Area (µV.s) ± S. D. (n=6)	% R.S.D
4.5	319475 ± 1478.64	0.4628

Table 4: Recovery Study Data

Name of Drug	% Level of Recovery	Test Amt. (µg/ml)	Amt. of Drug Spiked (µg/ml)	Total Std Amt. (µg/ml)	Total Amount Recovered (µg/ml)	% Recovery ± R.S.D. (n=3)
Valsartan	50	8	4	12	11.94	99.50 ± 0.3615
	100	8	8	16	15.98	99.87 ± 0.3587
	150	8	12	20	20.03	100.15 ± 0.2911
Nifedipine	50	3	1.5	4.5	4.47	99.33 ± 0.4431
	100	3	3	6	5.98	99.67 ± 0.3571
	150	3	4.5	7.5	7.53	100.40 ± 0.3347

Table 5: LOD and LOQ Data

Drug Name	VALSARTAN	NIFEDIPINE
Standard Deviation	1294	723
Slope	39992	11483
LOD (µg/ml)	0.1067	0.2078
LOQ (µg/ml)	0.3236	0.6298

Table 6: Application of HPLC Method to Synthetic Mixture

Drug Name	Amount in Synthetic Mixture (µg/ml)	Amount Found (µg/ml) ± S.D. (n=3)	% Assay ± R.S.D. (n=3)
VALSARTAN	8	8.03 ± 1688.82	100.37 ± 0.3848
NIFEDIPINE	3	2.98 ± 1404.87	99.33 ± 0.3091

Table 7: Robustness Data of VALSARTAN and NIFEDIPINE

Condition	Variation	VALSARTAN	NIFEDIPINE
		%Assay ± R.S.D (n=3)	%Assay ± R.S.D (n=3)
Flow Rate (1 ml ± 0.1 ml/ min)	0.9 ml/min	99.50 ± 0.5461	98.50 ± 0.3744
	1.0 ml/min	100.50 ± 0.5645	100.50 ± 0.4659
	1.1 ml/min	100.83 ± 0.6026	99.00 ± 0.4884
Detection Wavelength (257 nm ± 1 nm)	232 nm	99.67 ± 0.4705	99.67 ± 0.3877
	233 nm	100.50 ± 0.5645	100.50 ± 0.4659
	234 nm	101.00 ± 0.5910	99.50 ± 0.5735
Change in Mobile Phase Composition (%v/v/v)	69: 26: 05	99.33 ± 0.4301	98.00 ± 0.4093
	70: 25: 05	100.50 ± 0.5645	100.50 ± 0.4659
	70: 24: 06	100.66 ± 0.6495	98.50 ± 0.5310

Table 8: Summary of Validation Parameters

Sr. No.	Parameters	VALSARTAN	NIFEDIPINE
1	Linearity Range (µg/ml)	4-20	1.5-7.5
2	Regression Line Equation (y = mx + c)	y = 39992x+68009	y = 11484x-18115
3	Correlation Coefficient (R^2)	0.999	0.998
4	Intraday Precision (%RSD, n=3)	0.3584 – 0.5033	0.2983 – 0.4804
5	Interday Precision (% RSD, n=3)	0.4214 – 0.6017	0.4378 – 0.5492
6	Repeatability (% RSD, n=6)	0.5635	0.4628
7	LOD (µg/ml)	0.1067	0.2078
8	LOQ (µg/ml)	0.3236	0.6298
9	% Recovery Study (n=3)	99.50 – 100.15	99.33 – 100.40

RESULTS:

A Reverse Phase Column proposed as a suitable method for the determination of Valsartan and Nifedipine in Synthetic Mixture. The Chromatographic condition were optimized by changing the Mobile Phase Composition. Different ratios were experimented to optimize the Mobile Phase. Finally, **:** Methanol: Water: ACN (pH 3.7 adjusted with 10% Ortho Phosphoric Acid) (70:25:05 %v/v/v) as Mobile Phase which shows good resolution of Valsartan and Nifedipine peak. The wavelength of detection selected was 233 nm, as the drug shows optimized absorbance at this wavelength. By our proposed method Retention Time of Valsartan and Nifedipine were about 8.003 min and 5.290 min respectively and none of the Impurities were interfering in its assay.

DISCUSSIONS:

The statistical analysis of data and the drug recovery data showed that the method was simple, rapid, economical, sensitive, precise and accurate. It can thereby easily adopt for routine quality control analysis. The results of this analysis confirmed that the proposed method was suitable for determination of drug in Synthetic Mixture with virtually no interference of additives. Hence the proposed method can be successfully applied in estimation of Valsartan and Nifedipine in Synthetic Mixture.

CONCLUSION:

A simple, rapid, sensitive, accurate and precise RP-HPLC Method has been developed and validated for routine analysis of VALSARTAN and NIFEDIPINE in Synthetic Mixture. The RP-HPLC method is suitable for simultaneous estimation of VALSARTAN and NIFEDIPINE in Synthetic Mixture without interference of each other. The developed method was successfully applied in Synthetic Mixture. The proposed Method can be utilized for the routine analysis of VALSARTAN and NIFEDIPINE in Synthetic Mixture.

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