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Research

Analytical Method Development And Validation For Estimation Of Reserpine And Dihydralazine In Bulk And Tablet Dosage Form By Hplc

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Abstract

A Rapid and Precise Reverse Phase High Performance Liquid Chromatographic method has been developed for the validated of Reserpine and Dihydralazine, in its pure form as well as in tablet dosage form. Chromatography was carried out on X-Terra C18 (4.6 x 150mm, 5µm) column using a mixture of Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) as the mobile phase at a flow rate of 1.0ml/min, the detection was carried out at 212 nm. The retention time of the Reserpine and Dihydralazine was 2.090, 5.289 ±0.02min respectively. The method produce linear responses in the concentration range of 5-25mg/ml of Reserpine and 45-225mg/ml of Dihydralazine. The method precision for the determination of assay was below 2.0%RSD. The method is useful in the quality control of bulk and pharmaceutical formulations.

Keywords: Reserpine, Dihydralazine, RP-HPLC, validation.

INTRODUCTION

Chromatography
Introduction

The chromatography was discovered by Russian Chemist and botanist *Micheal Tswett* (1872-1919) who first used the term chromatography (colour writing derived from Greek for colour – Chroma, and write – graphein) to describe his work on the separation of coloured plant pigments into bands on a column of chalk and other material such as polysaccharides, sucrose and insulin.[1] "*Chromatography is a method in which the components of a mixture are separated on an adsorbent column in a flowing system*". The adsorbent material, or stationary phase, first described by Russian scientist named Tswett in 1906, has taken many forms over the years, including paper, thin layers of solids attached to glass plates, immobilized liquids, gels, and solid particles packed in columns. The flowing

component of the system, or mobile phase, is either a liquid or a gas. Concurrent with development of the different adsorbent materials has been the development of methods more specific to particular classes of analytes. In general, however, the trend in development of chromatography has been toward faster, more efficient. "In his early papers of Tswett (1906) stated that chromatography is a method in which the component of a mixture are separated on an adsorbent column in a flowing system.[2,3] Chromatography has progressed considerably from Tswett's time and now includes a number of variations on the basic separation process". "Chromatography is a physical method of separation in which the component to be separated are distributed between two phases of which in stationary while other moves in a definite direction (IUPAC)"[4]

Chromatographic Process

Chromatographic separations are based on a forced transport of the liquid (mobile phase) carrying the analyte mixture through the porous media and the differences in the interactions at analytes with the surface of this porous media resulting in different migration times for a mixture components. In the above definition the presence of two different phases is stated and consequently there is an interface between them. One of these phases provides the analyte transport and is usually referred to as the mobile phase, and the other phase is immobile and is typically referred to as the stationary phase.[5] A mixture of components, usually called analytes, are dispersed in the mobile phase at the molecular level allowing for their uniform transport and interactions with the mobile and stationary phases. High surface area of the interface between mobile and stationary phases is essential for space discrimination of different components in the mixture. Analyte molecules undergo multiple phase transitions between mobile phase and adsorbent surface. Average residence time of the molecule on the stationary phase surface is dependent on the interaction energy. For different molecules with very small interaction energy difference the presence of significant surface is critical since the higher the number of phase transitions that analyte molecules undergo while moving through the chromatographic column, the higher the difference in their retention.[6] The nature of the stationary and the mobile phases, together with the mode of the transport through the column, is the basis for the classification of chromatographic methods.[7,8]

Types of Chromatography

The mobile phase could be either a liquid or a gas, and accordingly we can subdivide chromatography into Liquid Chromatography (LC) or Gas Chromatography (GC). Apart from these methods, there are two other modes that use a liquid mobile phase, but the nature of its transport through the porous stationary phase is in the form of either (a) capillary forces, as in planar chromatography (also called Thin-Layer Chromatography, TLC), or (b) electro osmotic flow, as in the case of Capillary Electro Chromatography (CEC).[9,10]

MATERIALS AND METHODS

Reserpine & Dihydralazine Procured from Sura labs, Water and Methanol for HPLC from LICHROSOLV (MERCK), Acetonitrile for HPLC from Merck.

HPLC METHOD DEVELOPMENT

TRAILS

Preparation of standard solution

Accurately weigh and transfer 10 mg of Reserpine and Dihydralazine working standard into a 10ml of clean dry volumetric flasks add about 7ml of Methanol and sonicate to dissolve and removal of air completely and make volume up to the mark with the same Methanol. Further pipette 0.15ml of the above Reserpine and 0.1.35ml of Dihydralazine stock solutions into a 10ml volumetric flask and dilute up to the mark with Methanol.

Procedure

Inject the samples by changing the chromatographic conditions and record the chromatograms, note the conditions of proper peak elution for performing validation parameters as per ICH guidelines.

Mobile Phase Optimization

Initially the mobile phase tried was Methanol: Water and Water: Acetonitrile and Methanol: TEA Buffer: ACN with varying proportions. Finally, the mobile phase was optimized to Methanol: TEA Buffer: ACN in proportion 50:25:25 v/v respectively.

Optimization of Column

The method was performed with various columns like C18 column, Symmetry and Zodiac column. X-Terra C18 (4.6×150mm, 5µ) was found to be ideal as it gave good peak shape and resolution at 1ml/min flow.

OPTIMIZED CHROMATOGRAPHIC CONDITIONS

Instrument used	:	Waters HPLC with auto sampler and PDA Detector 996 model.
Temperature	:	Ambient
Column	:	X-Terra C18 (4.6×150mm, 5µ)
Buffer	:	Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the pH 4.5. Fliter and sonicate the solution by vaccum filtration and ultra sonication.
pH	:	4.5
Mobile phase	:	Methanol: TEA buffer: ACN (65:15:20v/v)
Flow rate	:	1ml/min
Wavelength	:	212 nm
Injection volume	:	10 µl
Run time	:	10 min

VALIDATION

PREPARATION OF BUFFER AND MOBILE PHASE

Preparation of Triethylamine (TEA) buffer (pH-4.5)

Dissolve 1.5ml of Ttiethyl amine in 250 ml HPLC water and adjust the p^H 4.5. Fliter and sonicate the solution by vaccum filtration and ultra sonication.

Preparation of mobile phase

Accurately measured 650 ml (65%) of Methanol, 150 ml of Triethylamine buffer (15%) and 200 ml of Acetonitrile (20%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration.

Diluent Preparation

The Mobile phase was used as the diluent.

RESULTS AND DISCUSSION

Optimized Chromatogram (Standard)

Mobile phase	:	Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20)
Column	:	X-Terra C18 (4.6×150mm, 5.0 µm)
Flow rate	:	1 ml/min
Wavelength	:	212 nm
Column temp	:	Ambient
Injection Volume	:	10 µl
Run time	:	10 minutes

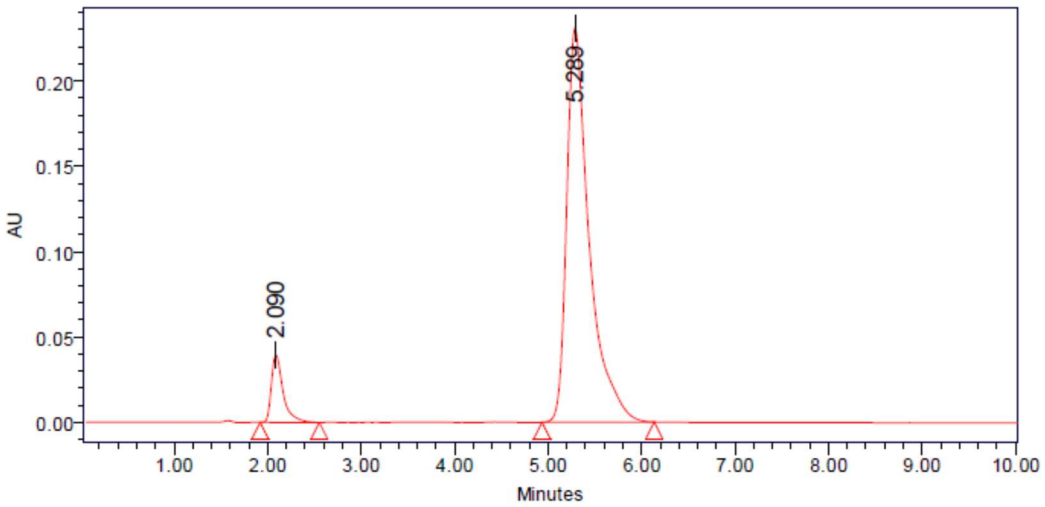

Fig 1: Optimized Chromatogram

Table 1: peak results for optimized

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Reserpine	2.090	372126	39690		1.70	5587
2	Dihydralazine	5.289	3864998	231194	9.80	1.77	5698

Observation: From the above chromatogram it was observed that the Reserpine and Dihydralazine peaks are well separated and they shows proper retention time, resolution, peak tail and plate count. So it's optimized trial.

Optimized Chromatogram (Sample)

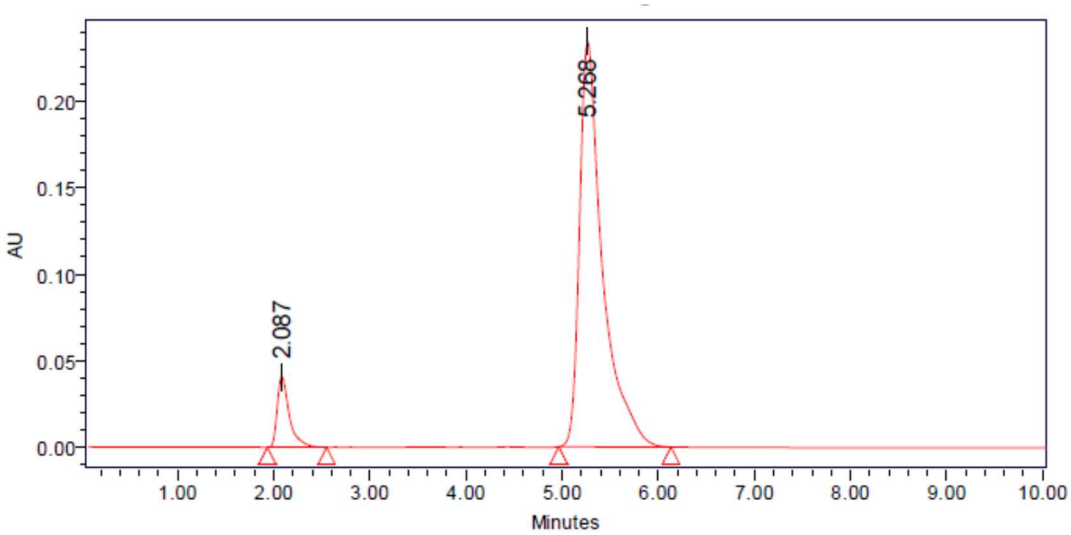

Fig 2: Optimized Chromatogram (Sample)

Table 2: Optimized Chromatogram (Sample)

S. No	Peak name	R_t	Area	Height	USP Resolution	USP Tailing	USP plate count
1	Reserpine	2.087	356547	41157		1.72	5557
2	Dihydralazine	5.268	3896493	234961	9.82	1.91	5804

Assay (Standard)

Table 3: Peak results for assay standard

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Reserpine	2.090	348126	39690		1.70	5587	1
2	Dihydralazine	5.289	3864998	231194	9.80	1.77	5628	1
3	Reserpine	2.089	352564	39990		1.66	5571	2
4	Dihydralazine	5.338	3881443	231044	9.93	1.83	5688	2
5	Reserpine	2.089	357976	40396		1.68	5530	3
6	Dihydralazine	5.327	3896952	231969	9.91	1.86	5712	3

Assay (Sample)

Table 4: Peak results for Assay sample

S no	Name	Rt	Area	Height	USP Resolution	USP Tailing	USP plate count	Injection
1	Reserpine	2.088	352290	40269		1.69	5516	1
2	Dihydralazine	5.276	3883794	231354	9.75	1.89	5677	1
3	Reserpine	2.087	356547	41157		1.72	5557	2
4	Dihydralazine	5.268	3896493	234961	9.82	1.91	5804	2
5	Reserpine	2.085	358914	40963		1.75	5489	3
6	Dihydralazine	5.262	3900103	233541	9.78	1.95	5790	3

$$\%ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

=355917/352888.7×10/60×60/0.072×99.7/100×0.0360/5×100
=100.5%
The % purity of Reserpine and Dihydralazine in pharmaceutical dosage form was found to be100.5%.

SYSTEM SUITABILITY

Table 5: Results of system suitability for Reserpine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Reserpine	2.090	342126	39690	5463	1.42
2	Reserpine	2.090	342426	39690	5576	1.42
3	Reserpine	2.089	342564	39990	5098	1.44
4	Reserpine	2.089	347976	40396	5143	1.43
5	Reserpine	2.085	352914	40963	5674	1.47
Mean			345601.2			
Std. Dev			4756.58			
% RSD			1.3			

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is suitable.*

Table 6: Results of system suitability for Reserpine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dihydralazine	5.289	3864998	231194	5786	1.46	9.80
2	Dihydralazine	5.289	3864998	232184	5908	1.47	9.81
3	Dihydralazine	5.338	3881443	231044	5487	1.48	9.81
4	Dihydralazine	5.327	3896952	231969	5032	1.40	9.83
5	Dihydralazine	5.262	3900103	233541	5389	1.43	9.82

Mean	3881699
Std. Dev	16802.33
% RSD	0.4

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

LINEARITY

Reserpine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33.3	5	134436
66.6	10	245571
100	15	371548
133.3	20	499024
166.6	25	619830

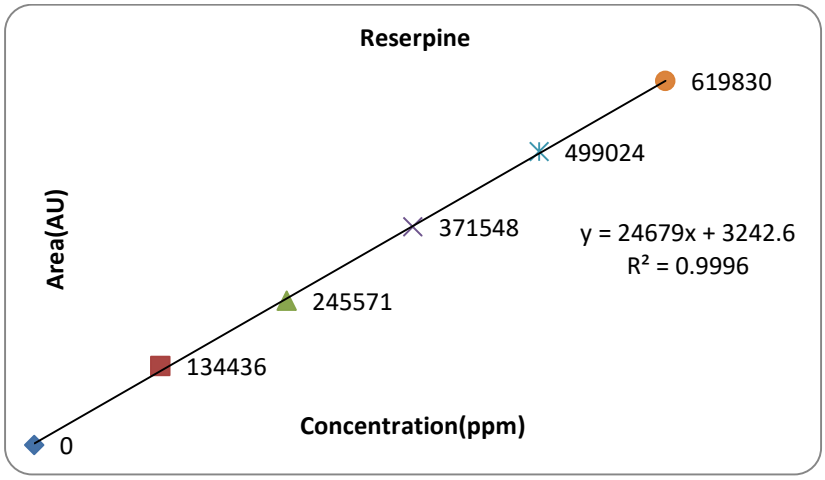

Fig 3: calibration graph for Reserpine

Dihydralazine

Concentration Level (%)	Concentration µg/ml	Average Peak Area
33	45	1330054
66	90	2728974
100	135	3917063
133	180	5300022
166	225	6412695

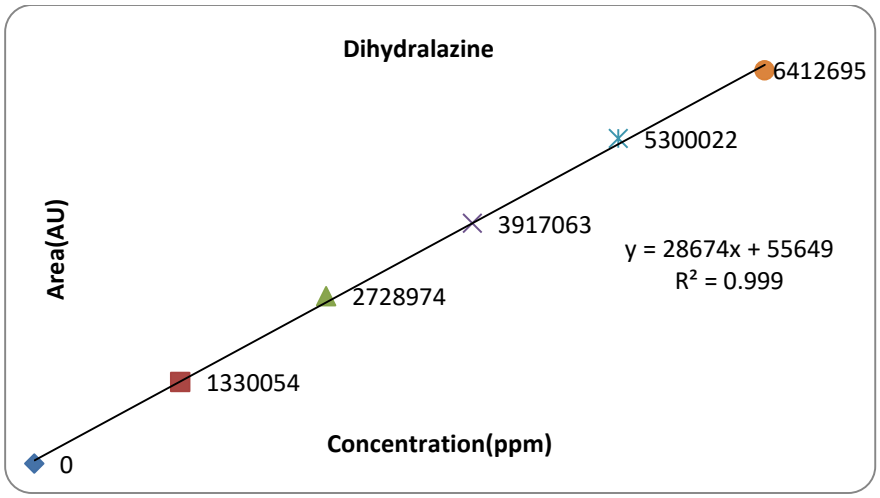

Fig 4: Calibration graph for Dihydralazine

REPEATABILITY

Table 7: Results of repeatability for Reserpine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Reserpine	2.086	362266	41697	5081.3	1.8
2	Reserpine	2.083	364902	41402	5144.1	1.8
3	Reserpine	2.083	366870	41540	5118.1	1.8
4	Reserpine	2.081	367273	42256	5147.3	1.8
5	Reserpine	2.081	368101	42143	5101.8	1.8
Mean			365882.4			
Std. Dev			2338.4			
% RSD			0.6			

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Table 8: Results of method precession for Dihydralazine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dihydralazine	5.178	3903548	240181	5988.3	2.0	9.8
2	Dihydralazine	5.199	3905819	235523	5856.3	2.0	9.7
3	Dihydralazine	5.235	3916120	238578	5930.2	2.0	9.9
4	Dihydralazine	5.202	3916542	238814	5936.9	2.0	9.8
5	Dihydralazine	5.206	3920943	241006	5040.0	2.0	9.5
Mean			3912594.4				
Std. Dev			7507.6				
% RSD			0.2				

- *%RSD for sample should be NMT 2*
- *The %RSD for the standard solution is below 1, which is within the limits hence method is precise.*

Intermediate precision
Day-1

Table 9: Results of Intermediate precision for Reserpine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Reserpine	2.083	369246	42277	5537.8	1.6
2	Reserpine	2.083	370766	42708	5561.8	1.6
3	Reserpine	2.089	370840	42065	5489.3	1.6
4	Reserpine	2.083	370840	42065	5489.3	1.6
5	Reserpine	2.082	371041	42568	5583.2	1.8
6	Reserpine	2.080	371386	42211	5533.2	1.8
Mean			370686.5			
Std. Dev			740.7369			
% RSD			0.19			

- *%RSD of five different sample solutions should not more than 2*

Table 10 : Results of Intermediate precision for Dihydralazine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dihydralazine	5.229	3743003	242955	5269.7	2.2	10.2
2	Dihydralazine	5.203	3845359	242255	5100.5	2.1	10.0
3	Dihydralazine	5.133	3885014	242854	5127.6	2.1	10.0
4	Dihydralazine	5.229	3743003	242955	5269.7	2.2	10.2
5	Dihydralazine	5.151	3722513	240346	5048.8	1.5	9.9
6	Dihydralazine	5.112	3728789	237638	5997.2	1.6	9.9
Mean			3777947				
Std. Dev			69194.4				
% RSD			1.8				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

Day 2

Table 11: Results of Intermediate precision Day 2 for Reserpine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing
1	Reserpine	2.078	370979	42978	3083.0	1.9
2	Reserpine	2.082	371041	42568	3583.2	1.8
3	Reserpine	2.080	371386	42211	3533.2	1.8
4	Reserpine	2.089	369246	42277	1537.8	1.6
5	Reserpine	2.083	370840	42065	1489.3	1.6
6	Reserpine	2.089	369246	42277	1537.8	1.6
Mean			370456.3			
Std. Dev			954.6004			
% RSD			0.25			

- *%RSD of five different sample solutions should not more than 2*

Table 12: Results of Intermediate precision for Dihydralazine

S no	Name	Rt	Area	Height	USP plate count	USP Tailing	USP Resolution
1	Dihydralazine	5.077	3841404	246818	5208.0	2.1	10.1
2	Dihydralazine	5.151	3885014	242854	5127.6	2.1	10.0

3	Dihydralazine	5.112	3743003	242955	5269.7	2.2	10.2
4	Dihydralazine	5.133	3743003	242955	5269.7	2.2	10.2
5	Dihydralazine	5.203	3885014	242854	5127.6	2.1	10.0
6	Dihydralazine	5.133	3743003	242955	5269.7	2.2	10.2
Mean			3806740				
Std. Dev			71613.47				
% RSD			1.8				

- *%RSD of five different sample solutions should not more than 2*
- *The %RSD obtained is within the limit, hence the method is rugged.*

ACCURACY

The accuracy results for Reserpine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	192446.6	7.5	7.4	98.6	
100%	374222	15	14.8	98.66	98.7%
150%	555891.3	22.5	22.3	99.1	

The accuracy results for Dihydralazine

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	2001752	67.5	67.3	99.7	
100%	3927797	135	134.8	99.8	99.7%
150%	5858665	202.5	202.1	99.8	

- *The percentage recovery was found to be within the limit (98-102%).*

The results obtained for recovery at 50%, 100%, 150% are within the limits. Hence method is accurate.

Robustness

Table 13: Results for Robustness

Reserpine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	372126	2.090	5587	1.70
Less Flow rate of 0.9 mL/min	356765	2.736	5432	1.82
More Flow rate of 1.1 mL/min	342356	1.673	5644	1.91
Less organic phase	312434	2.736	5098	1.82
More organic phase	305623	1.673	5123	1.91

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

Dihydralazine

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	3864998	5.289	5698	1.77
Less Flow rate of 0.9 mL/min	3546737	6.746	5546	1.88
More Flow rate of 1.1 mL/min	3857216	4.032	5124	1.91
Less organic phase	3810347	6.746	5034	1.88
More organic phase	3875642	4.032	5612	1.91

The tailing factor should be less than 2.0 and the number of theoretical plates (N) should be more than 2000.

CONCLUSION

In the present investigation, a simple, sensitive, precise and accurate RP-HPLC method was developed for the quantitative estimation of Reserpine and Dihydralazine in bulk drug and pharmaceutical dosage forms. This method was simple, since diluted samples are directly used without any preliminary chemical derivatisation or purification steps. Reserpine and Dihydralazine was freely soluble in ethanol, methanol and sparingly soluble in water. Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was chosen as the mobile phase. The solvent system used in this method was economical. The %RSD values were within 2 and the method was found to be precise. The results expressed in Tables for RP-HPLC method was promising. The RP-HPLC method is more sensitive, accurate and precise compared to the Spectrophotometric methods. This method can be used for the routine determination of Reserpine and Dihydralazine in bulk drug and in Pharmaceutical dosage forms.

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REFERENCES

1. Dr. Kealey and P.J Haines, Analytical Chemistry, 1st edition, Bios Publisher, 2002, PP 1-7.
2. A.BraithWait and F.J.Smith, Chromatographic Methods, 5th edition, Kluwer Academic Publisher, 1996, PP 1-2.
3. Andrea Weston and Phyllisr. Brown, HPLC Principle and Practice, 1st edition, Academic press, 1997, PP 24-37.
4. Yuri Kazakevich and Rosario Lobrutto, HPLC for Pharmaceutical Scientists, 1st edition, Wiley Interscience A JohnWiley & Sons, Inc., Publication, 2007, PP 15-23.
5. Chromatography, (online). URL:http://en.wikipedia.org/wiki/Chromatography.
6. Meyer V.R. Practical High-Performance Liquid Chromatography, 4th Ed. England, John Wiley & Sons Ltd, 2004, PP 7-8.
7. Sahajwalla CG a new drug development, vol 141, Marcel Dekker Inc., New York, 2004, PP 421–426.
8. Introduction to Column. (Online), URL: http://amitpatel745.topcities.com/index_files/study/column care.pdf
9. Draft ICH Guidelines on Validation of Analytical Procedures Definitions and terminology. Federal Register, vol 60. IFPMA, Switzerland, 1995, PP 1126.
10. Code Q2B, Validation of Analytical Procedures; Methodology. ICH Harmonized Tripartite Guidelines, Geneva, Switzerland, 1996, PP 1- 8.
11. Snyder LR practical HPLC method development, 2nd edition. John Wiley and sons, New York, 1997, PP 180-182.