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Research article

RP-HPLC METHOD AND IT'S VALIDATION FOR ANALYSIS OF RESERPINE AND DIHYDRALAZINE IN PURE AND PHARMACEUTICAL DOSAGE FORM

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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 produces 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, PDA detection, Method validation.

INTRODUCTION

Dihydralazine is chemically 1,4-Dihydrazinophthalazine. An antihypertensive agent with actions and uses similar to those of Hydralazine. (From Martindale, The Extra Pharmacopoeia, 30th ed, p354) [1-3]. Reserpine is described chemically as Methyl 11,17 α-dimethoxy-18β-[(3,4,5-trimethoxy benzoyl)oxy]-3β,20α-yohimban-16β-carboxylate. The empirical formula is $C_{33}H_{40}N_2O_9$ .The structural formula is shown infig.1 [1-3].

Reserpine is a white or slightly yellow, small crystals or crystalline powder, darkening slowly on exposure to light. It is practically insoluble in water, very slightly soluble in ethanol (96 per cent). It contains not less than 97.0% and not more than 101.0% of $C_{33}H_{40}N_2O_9$

Reserpine is used with or without other medications to treat high blood pressure (hypertension). Lowering high blood pressure helps prevent strokes, heart attacks and kidney problems, it works by decreasing certain substances in the body (such as norepinephrine), this causes the blood vessels to relax so that blood can flow more easily and also slows the heart rate, these effects help to lower blood pressure.

The significance of this study due to the simultaneous determination of three drug substances (and Reserpine) in one drug product used for heart diseases which should be very precise. The objective of this study is to develop a new, rapid, precise and sensitive RP-HPLC method for the simultaneous determination Dihydralazine and Reserpine in pharmaceutical dosage forms as per ICH guidelines. The validation procedure followed the guidelines of USP 34 [4-7].

MATERIALS AND METHODS

Chemicals and reagents

All reagents and chemicals were highly-pure, phosphoric acid, HPLC grade acetonitrile and HPLC grade methanol were obtained by Merck India, ammonium acetate was obtained by Dihydralazine procured from Dr.Reddys Labs Hyderabad. Reserpine

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procured from Vimta Pharma Labs Hyderabad, Water was deionized and passed through Millie Q system, Millie pore (USA).

Equipments

HPLC model Waters 2695, equipped with a degasser and column oven, and an auto sampler with injection volume ranges of 1.0-100.0 µl, UV detection at 190-900 nm, pump range of flow rate 0.0-10.0 ml/min, separation was achieved on a BDS Hypersil.

Chromatographic conditions

The mobile phase consists of a mixture of Methanol: TEA buffer: ACN (65:15:20v/v) to a pH-4.5 and filtered through 0.45 µm nylon membrane filter before use. The injection volume was 20 µl with a flow rate 1.0 ml/min and detection wavelength 212 nm having ambient condition.

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.

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: ACNin 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 vaccumfiltration 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.

VALIDATION PARAMETERS

SYSTEM SUITABILITY

1. Accurately weigh and transfer 10 mg of Reserpine and 10mg of Dihydralazineworking standard into a 10ml of clean dry volumetric flasks add about 7mL of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)**.** Further pipette 0.15ml of the above Reserpine and 1.35ml of Dihydralazine stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent. The results are shown table 1 &2.

Method validation

Validation of an analytical method is the process that establishes, by laboratory studies, that the performance characteristics of the method meet the requirements for the intended analytical applications. The validation of this suggested method is according to ICH guidelines. Method validation is the process of demonstrating that analytical procedures are suitable for their intended use, therefore the objective of this analytical procedure validation is to demonstrate that it is suitable for its intended purpose.

Specificity

The ICH documents define specificity as the ability to assess unequivocally the analyte in the presence of components that may be expected to be present, such as impurities, degradation products, and matrix components. Analytical method was tested for specificity to measure accurately quantitate Reserpine and Dihydralazine in drug product Fig.3.

Linearity

Linearity was studied by preparing standard solution at five different concentration levels. The linearity range was found to be 5-25 µg/ml for reserpine and 45 -245 for dihydralzine respectively. 20 µl of each solution was injected into chromatograph. Peak areas were recorded for all the chromatogram. Calibration curve was constructed by plotting the ratio of area of studied component to area of Caffeine. The linearity curves of Dihydralazine and Reserpine are shown in fig. 4-5 and Table 3 & 4.

Accuracy and precision

Accuracy and precision of the method were established by five replicate injections of the standard solution containing Dihydralazine and Reserpine in the same day. The confidence limit CL (confidence level = 95%, n-1=4 and t=2.78), recovery REC% and RSD% were calculated and presented in table 1. From the data obtained, this developed RP-HPLC method was found to be accurate and precise. The precision and accuracy data values shown in Table 5,6,7 and 8.

Table 1. 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.			
Std. Dev			4756.58			
% RSD			1.3			

Table 2. 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				

Table 3. Linearity data of Reserpine

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

Table 4. Linearity data of Dihydralazine

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

Table 5. Results of Intermediate precision

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			

Table 6. Results of Intermediate precision for Dihydralazine

S no	Name	Rt	Peak 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				

Table 7. The Accuracy results for Reserpine

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

Table 8. 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	99.7%
100%	3927797	135	134.8	99.8	
150%	5858665	202.5	202.1	99.8	

Fig 1. Chemical structure of Dihydralazine

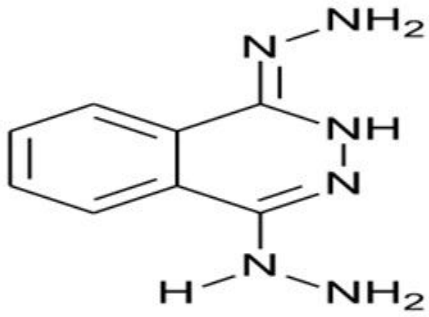


Fig 2. Chemical structure of Reserpine

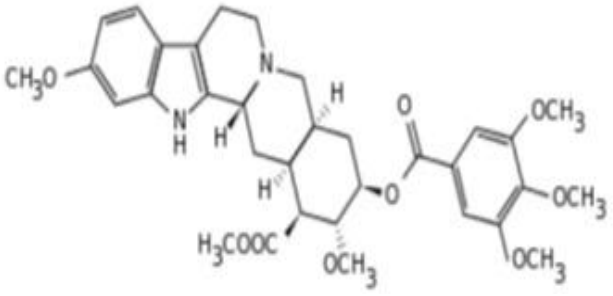


Fig 3. Typical Chromatogram of mixed standard solution

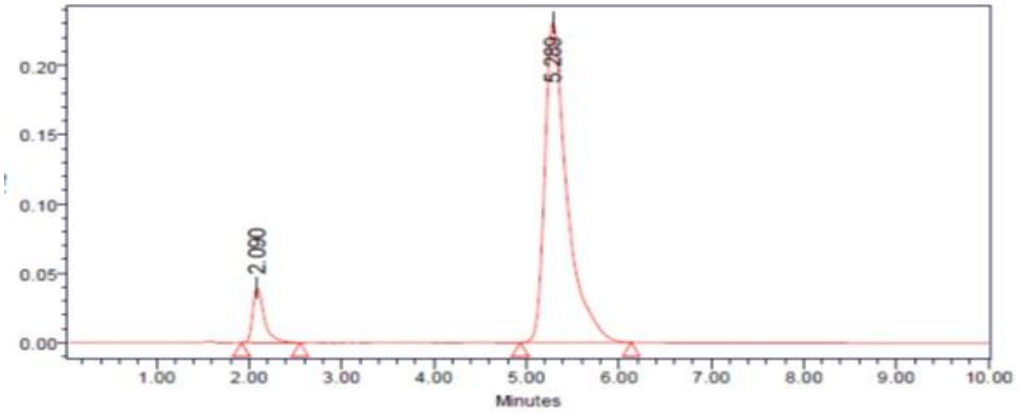


Fig 4. calibration graph for Reserpine

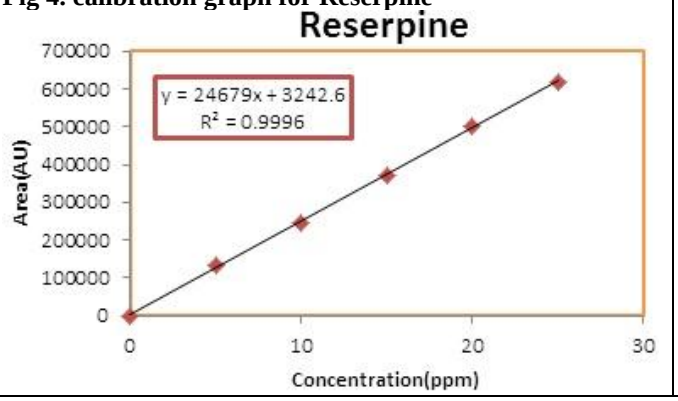
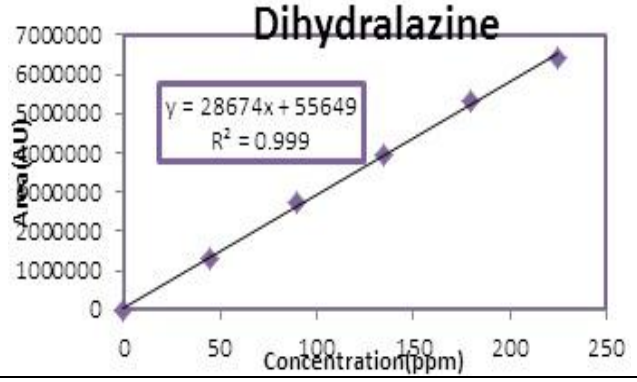


Fig 5. Calibration graph for Dihydralazine



SUMMARY AND CONCLUSION

The analytical method was developed by studying different parameters. First of all, maximum absorbance was found to be at 212 nm and the peak purity was excellent. Injection volume was selected to be 10µl which gave a good peak area. The column used for study was X-Terra C_{18} because it was giving good peak. Ambient temperature was found to be suitable for the nature of drug solution. The flow rate was fixed at 1.0ml/min because of good peak area and satisfactory retention time. Mobile phase is Methanol: TEA Buffer pH 4.5: Acetonitrile (65:15:20) was fixed due to good symmetrical peak. So, this mobile phase was used for the proposed study. Run time was selected to be 10 min because analyze gave peak around 2.090, 5.289 ±0.02min respectively and also to reduce the total run time. The percent recovery was found to be 98.0-102 was linear and precise over the same range. Both system and method precision was found to be accurate and well within range. The analytical method was found linearity over the range 5-25mg/ml of Reserpine and 45-225 mg/ml of Dihydralazine of the target concentration. The analytical passed both robustness and ruggedness tests. On both cases, relative standard deviation was well satisfactory.

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 inTablesfor 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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