International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.11 No.03, pp 192-209, 2018

Stability Indicating RP-HPLC Method for Simultaneous Estimation of Reserpine, Dihydralazine Sulphate and Hydrochlorothiazide in Bulk and Pharmaceutical Dosage forms

Vellaturi Padmaja*[1] , Abdul Rahaman[2], K.Shanta Kumari[3], G.Seetha Kumari[4]

[1,2,3,4]**Nirmalacollege of Pharmacy, Atmakuru, Mangalagiri, Guntur district-522 503,India.**

Abstract : A simple, specific and accurate RP-HPLC method was developed for the simultaneous estimation of Reserpine, Dihydralazine sulphate and Hydrochlorothiazide in tablet dosage forms. A reversed phase Phenomenexluna C18, 250mm × 4.6 mm, 5µm and UV-Visible detector with mobile phase consisting of Acetonitrile and buffer 70:30(v/v) were used. Empower version 2 software was used. The flow rate was 1.0ml /min and effluents were monitored at 240nm. The retentiontime for Reserpine, Dihydralazine sulphate and Hydrochlorothiazide in tablet formulation were found to be 8.4min, 2.2min, and 4.7min respectively. The method was validated according to ICH guidelines for specificity, LOD, LOQ, Precision, Accuracy, Linearity, Ruggedness and Robustness. The method showed good reproducibility and recovery with %RSD less than 2.

Keywords : Reserpine , Dihydralazine sulphate, Hydrochlorothiazide, RP-HPLC, validation as per ICH guidelines, stability indicating.

Introduction :

Combination therapy or poly therapy is therapy that uses more than one medication. Typically, these terms refer to using multiple therapies to treat a single disease. [1]Poly therapy is a related term, referring to the use of multiple medications.[2]The combination of Reserpine, Dihydralazine sulphate and Hydrochlorthiazide are used for the treatment of Hypertension. [3]Mainly the Reserpine which lowers the blood pressure to prevent strokes, heart attacks and kidney problems.[4]The Hydrochlorthiazide is a diuretic which reduces the reabsorption of electrolytes from the renal tubules.[5]Reserpine (also known by trade names Raudixin, Serpalan, Serpasil) is an indole alkaloid, antipsychotic, and antihypertensive drug that has been used for the control of high blood pressure and for the relief of psychotic symptoms.[6]Biochem/physiol Actions. Dihydralazinesulfate is a vasodilator and an antihypertensive agent. It relaxes arterial smooth muscle by inhibiting the accumulation of intracellular free calcium. By relaxing vascular smooth muscle, vasodilators act to decrease peripheral resistance. The methods are published for individual drugs and also for 2 drugs i.e Dihydrochlorothiazide and Hydralazine by RP-HPLC method. Then there is no any new method developed for simultaneous estimation of Reserpine, Dihydralazine sulphate and Hydrochlorothiazide in combination form by RP-HPLC method.

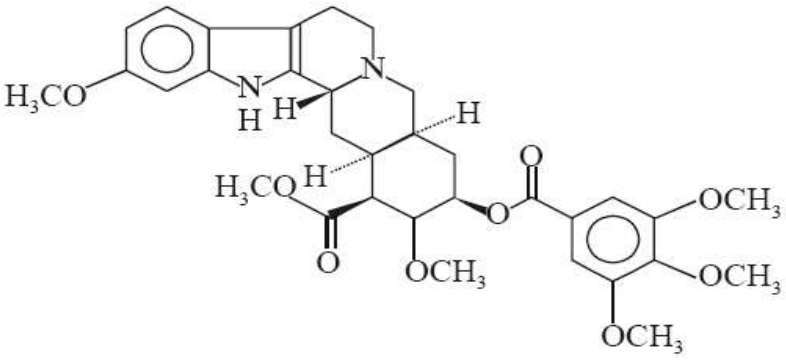
International Journal of ChemTech Research, 2018,11(03): 192-209

DOI : http://dx.doi.org/10.20902/IJCTR.2018.110322

Drug profile of 3 drugs :

1.Reserpine :

Structure:



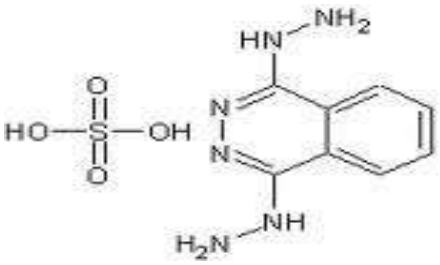
Reserpine

IUPAC name:

Methyl (3β,16β,17α,18β,20α)-11,17-dimethoxy-18-[(3,4,5-trimethoxybenzoyl)oxy]yohimban-16-carboxylate.

2.Dihydralazine sulphate:

Structure :

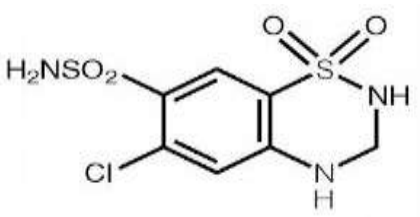


IUPACname:

4-hydrazinyl-1-hydrazinylidene-1,2-dihydropthalazine sulphate

3. **Hydrochlorothiazide :**

Structure :



IUPAC name:

6-chloro-1,1-dioxo-3,4-dihydro-2H-1,2,4-benzothiadiazine-7-sulfonamide**.**

Materials and methods:

Drug samples:

Reserpine,Dihydralazine sulphate and Hydrochlorothiazide pure drugs were procured from ICON laboratories Vijayawada, India . HPLC gradeAcetonitrile, HPLC grade water, Orthophosphoric acid were purchased from Merk and Rankem, India.

Instrumentation:

Instrument	Specifications
HPLC	Waters, 2695 separation module
Software	Empower, version 2.0
Detector	UV- visible detector
Analytical balance	Shimadzu

Optimised chromatographic conditions:

By performing the trails the chromatographic conditions were optimised as follows

Buffer : 1ml of Orthophosphoric acid was taken in 1litre of water
Mobile phase : Mixed Acetonitrile and buffer in the ratio 70:30%v/v
Diluents : Used mobile phase as diluent
Column : PhenomenexlunaC18(250×4.6mm)
Flow rate : 1ml/min
Wavelength : 240nm
Injectionvolume:20µl
ColumnTemperature : Room temperature

Standard solutionpreparation :

Solution A:(Reserpine)

Accuratelyweighed 0.1mg of Reserpine working standard into a 100ml volumetric flask. Added 70ml of diluentand sonicated to dissolve and then the volume was made up to mark with diluent.

Solution B:(Dihydralazine sulphate):

Accurately weighed10mg of Dihydralazine sulphate working standard into a 100ml volumetric flask. Added 70ml of diluent ,and sonicated to dissolve thenthe volume is made up to the mark with diluent.

Solution C:(Hydrochlorothiazide):

Accurately weighed10mg of Hydrochlorothiazide working standard in to a 100ml volumetric flask. Added 70 ml of diluent,and sonicated to dissolve then the volume is made up to the mark with diluent.

Sample preparation:

Reserpine- 0.1mg
Dihydalazine sulphate- 10mg,
Brand name :Adelphane
Hydrochlorothiazide -10mg.

Weighed 10 tablets and crushed to powder then 1 tablet equivalent powder was taken into a 250ml volumetric flask. 200ml of diluent was added, and sonicated to dissolve thenthe volume is made up to the mark. Further 5ml of this sample solution is diluted to 100ml with the diluent. It is filtered through 0.45µ nylon syringe filter.

Procedure:

20µl of standard preparation was injected five times and the system suitability parameters were noted from the recorded chromatogram. Then the sample solution was injected 5 times and the peak responses for the Reserpine ,Dihydralazine sulphate and Hydrochlorothiazide were noted from chromatograms and calculated the content of these drugs in the sample.

Table 1:

Drug	Labelled amount (mg)	Amount present(mg)	% assay
Reserpine	0.1	0.10	100.4
Dihydralazine sulphate	10	10.40	100.6
Hydrochlorothiazide	10	9.8	99.8

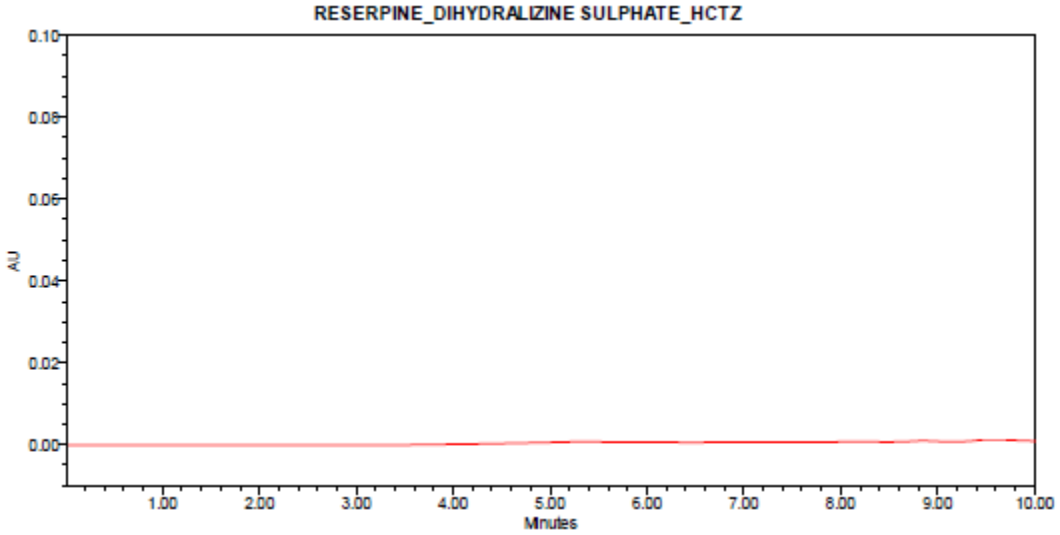


Fig1: A representative chromatogram of blank

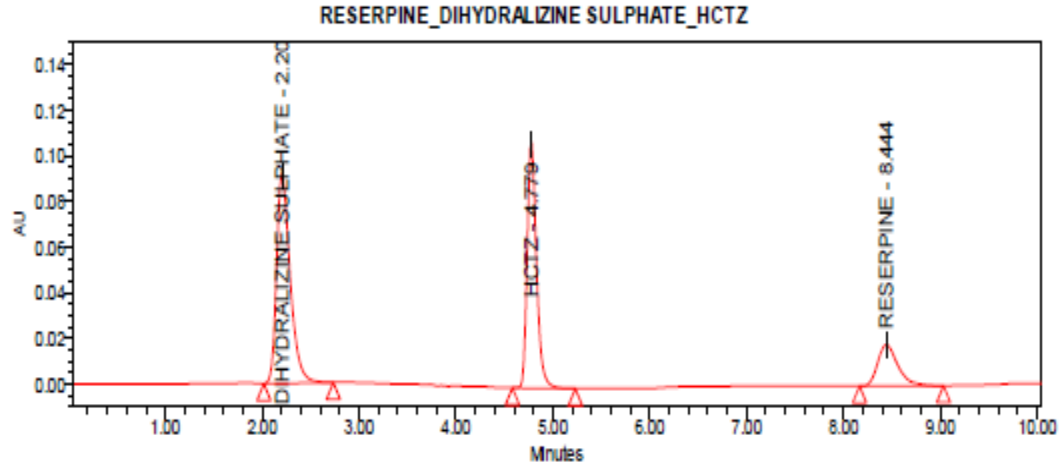


Fig2 : A representative chromatogram of standard

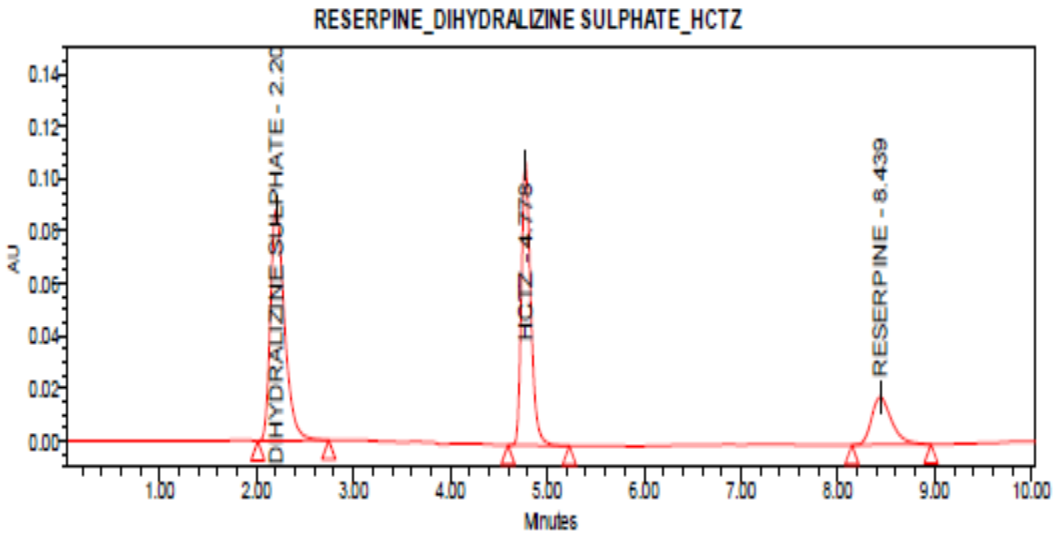


Fig3 : A representative chromatogram of sample

Method validation:

Table 2 :System suitability parameters :

S.NO	Name of the sample	USP plate count (n=5)	USP tailing (n=5)
1	Reserpine	11630	1.31
2	Dihydralazine sulphate	11637	1.48
3	Hydrochlorothiazide	12514	1.28

Observation :

From the system suitability studies it is observed that all parameters are within limits. Hence it is concluded that the instrument, reagents,and column are suitable to perform the assay.

Linearity:

Linearity of detector response for Reserpine, Dihydralzine sulphateand Hydrochlorothiazide was established for 6 concentrations ranging from 1 µg/ mlto 15 µg/ ml, 1µg/ml to 15 µg/ml, 100 µg/ml to 750 µg/ml of the target concentration. The final concentration of each solution in µg/ml was calculated and plotted against area response. The slope, y-intercept, correlation coefficient (R) were calculated. The linearity data was given in below table:

Table 3: Linearity data for Reserpine, Dihydralazine sulphate, and Hydrochlorthiazide

Drug name	Concentrationin µg/ml	Peak area
Reserpine	0	0
	1.82	238088
	3.23	493708
	4.04	629046
	4.85	778489
	6.87	1050185
	8.89	1373644
	10.50	1579270
	15.150	2338575

Dihydralazine sulphate	0	0
	1.87	189900
	3.33	390364
	4.16	483925
	4.99	579119
	7.07	856703
	8.32	102940
	9.57	119127
	15.600	189410
Hydrochlorothiazide	0	0
	1.84	141368
	3.26	278260
	4.08	374878
	4.90	464067
	6.94	650805
	8.16	755565
	10.00	956220
	15.300	1485674

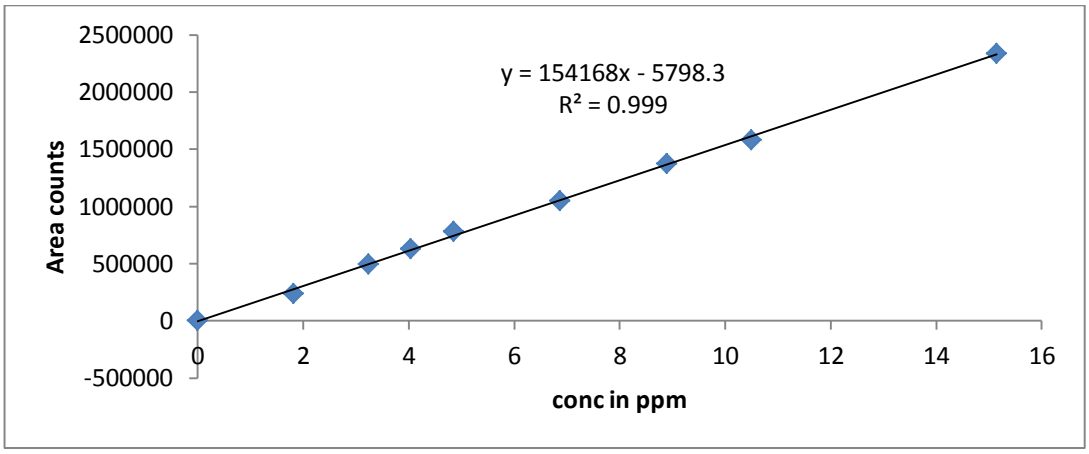


Fig 4:Linearity graph for Reserpine

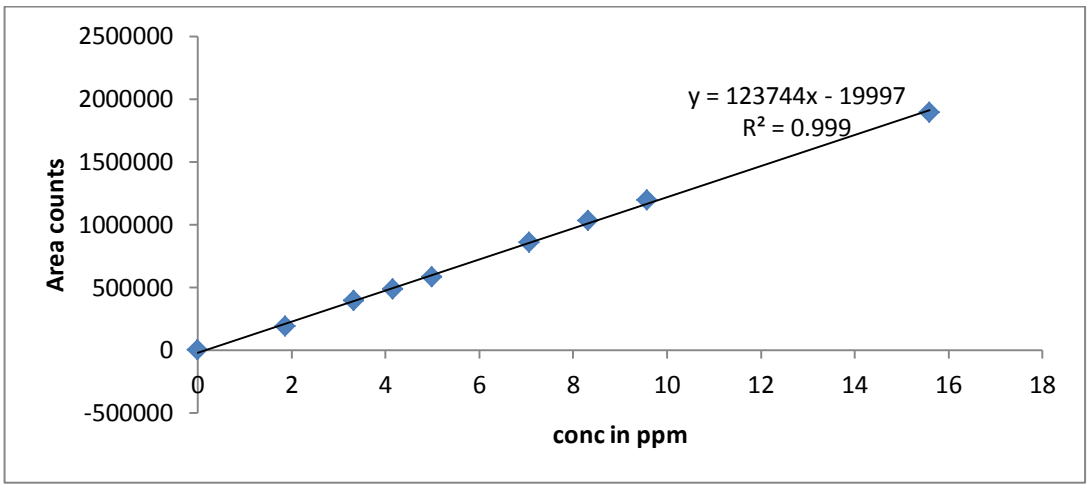


Figure5 : Linearity graph for Dihydralazine sulphate

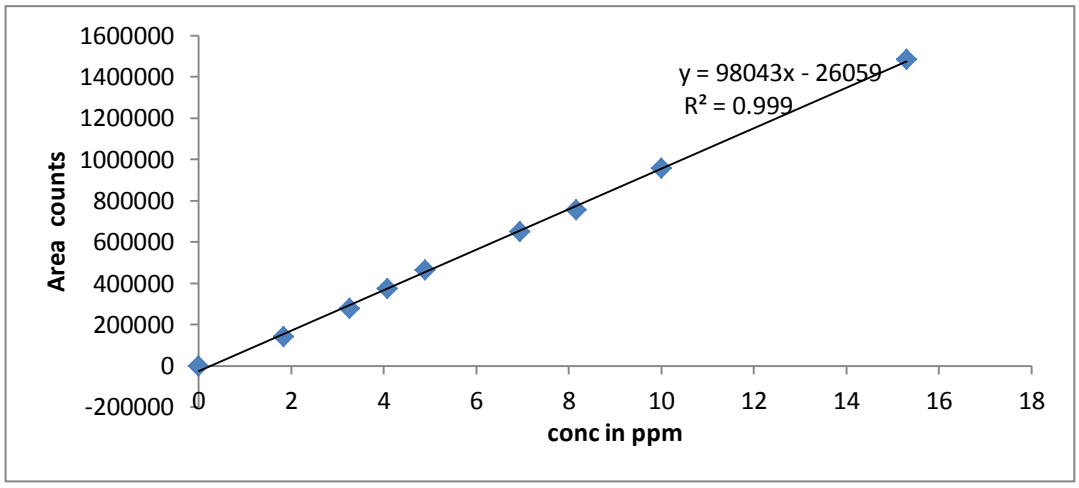


Figure 6:Linearity graph of Hydrochlorothiazide

Observation:

Results meet the established acceptance criteria where the correlation coefficient is found to be0.999.

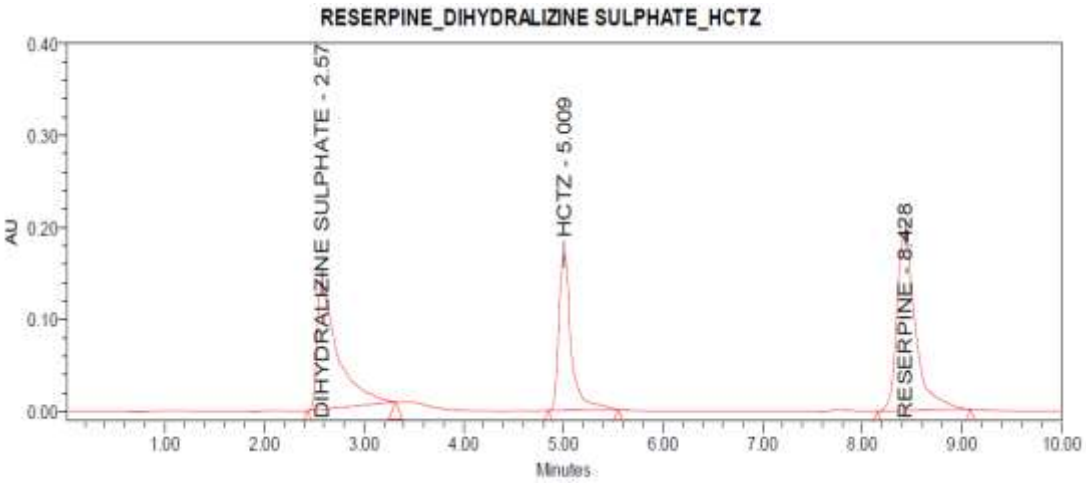


Fig 7: Chromatogram level of linearity

Accuracy:

The Accuracy was conducted for Reserpine, Dihydralazinesulphate and Hydrochlorothiazide. Assay in Triplicate (50%, 100%, and 150%) as per test method with equivalent amount of drug containing Reserpine, Dihydralazinesulphate and Hydrochlorothiazide into each volumetric flask, for each spike level to get the concentration of Reserpine, Dihydralazinesulphate and Hydrochlorothiazide equivalent to 50%, 100%, and 150% of the labelled amount as per the test method. The average % recovery was calculated.

Table-4 : Accuracy results of Reserpine by HPLC

S.No.	Accuracy	Amount Added (mg)	Area	Amt recovered	% Recovery	Results
1	50%	1.17	602536	119.19	100.2	Mean=100.2
2	50%	1.16	603163	119.32	99.8	SD=0.40
3	50%	1.16	605818	119.84	100.6	%RSD=0.440
1	100%	2.43	1258883	249.03	100.1	Mean=100.2
2	100%	2.42	1249694	247.21	99.9	SD=0.31

3	100%	2.41	1247854	246.85	100.5	%RSD=0.310
1	150%	4.78	2474621	489.53	100.2	Mean=100.4
2	150%	4.76	2474236	489.45	100.4	SD=0.20
3	150%	4.78	2879771	489.04	100.6	%RSD=0.200
					Mean=100.3 SD=0.115 %RSD=0.11	

Table-5: Accuracy results of Dihydralazine sulphate by HPLC

S.No.	Accuracy	Amount Added (mg)	Area	Amt recovered	% Recovery	Results
1	50%	25.8	480906	26.02	100.9	Mean=100.5
2	50%	26.20	487212	26.36	100.6	SD=0.38
3	50%	26.40	488617	26.43	100.1	%RSD=0.380
1	100%	52.20	969818	52.47	100.5	Mean=100.6
2	100%	51.60	962912	52.09	100.9	SD=0.260
3	100%	51.90	963942	52.15	100.5	%RSD=0.260
1	150%	98.50	1836079	99.33	100.8	Mean=100.7
2	150%	98.60	1838943	99.48	100.9	SD=0.32
3	150%	99.20	1839604	99.52	100.3	%RSD=0.310
					Mean=100.6 SD=0.1 %RSD=0.1	

Table-6: Accuracy results of hydrochlorthiazide by HPLC

S.No.	Accuracy	Amount Added (mg)	Area	Amt recovered	% Recovery	Results
1	50%	24.7	344921	24.86	100.6	Mean=100.3
2	50%	25.2	348763	25.14	99.8	SD=0.50
3	50%	24.6	343316	24.75	100.6	%RSD=0.500
1	100%	49.1	685905	49.44	100.7	Mean=100.1
2	100%	49.1	680786	49.08	100.0	SD=0.49
3	100%	49.3	682231	49.18	99.8	%RSD=0.490
1	150%	97.80	1316730	98.16	100.4	Mean=100.3
2	150%	98.20	1369154	98.7	100.5	SD=0.19
3	150%	98.40	1366916	98.54	100.1	%RSD=0.180
					Mean =100.2 SD=0.115 %RSD=0.11	

Observation:

The %recovery results indicating that the test method has an acceptable level of accuracy.

Precision:

1. System precision:

For injection repeatability, six injections from the same standard preparations were made and the relative standard deviation for the replicate injections was calculated. The readings of system precision were given in below tables:

Table-7: System precision values of Reserpine by HPLC

S. No.	RT(min)	Area	USP Plate count	USP tailing
1	8.407	259066	12035	1.32
2	8.407	258944	12031	1.32
3	8.407	258269	12049	1.31
4	8.441	254976	11588	1.25
5	8.444	257799	11117	1.35
6	8.453	256746	10990	1.34
		Mean=257633 %RSD=0.603		

Table- 8: System precision values of Dihydralazine sulphate by HPLC

S No.	RT(min)	Area	USP Plate count	USP tailing
1	2.235	941686	1.50	1.50
2	2.235	9490991	1.50	1203
3	2.235	9392011	1.49	1204
4	2.221	946578	1.48	1158
5	2.218	945217	1.52	1111
6	2.216	9490169	1.43	1099
		Mean=942307 %RSD=0.311		

Table-9: System precision values of Hydrochlorthiazide by HPLC

S.No	RT(min)	Area	USP Plate count	USP tailing
1	4.768	722455	1.27	12458
2	4.768	721977	1.27	12460
3	4.768	721691	1.26	12461
4	4.780	717938	1.31	12699
5	4.783	722739	1.28	12639
6	4.782	725180	1.35	12368
		Mean=721997 %RSD=0.325		

Observation:

From the system precisions studies it is observed that all the parameters like %RSD of retention time and peak areas are within limits

2. Method Precision: (Reproducibility)

Six individual preparations of Reserpine, Dihydralzinesulphate and Hydrochlorothiazide drug substance were prepared with a target concentration of aboutReserpine-0.10ppm, Dihydralazinesulphate-10.01ppm, Hydrochlorothiazide -10.01ppm.

Table-10: Method precision values of Reserpine by HPLC

S No.	RT(min)	Area	USP Plate count	USP tailing
1	7.997	1251130	12186	1.43
2	7.996	1249896	13082	1.38
3	7.841	1257277	12885	1.29
4	7.880	1256499	8159	1.25
5	7.879	1253805	9307	1.24
6	7.993	1259187	11186	1.22
		Mean=1254632 %RSD=0.291		

Table 11:Method precision values of Dihydralazine sulphate by HPLC

S.No	RT(min)	Area	USP Plate count	USP tailing
1	2.632	881948	1382	2.03
2	2.608	887765	1332	2.04
3	2.561	888735	1556	2.14
4	2.570	884041	1640	1.97
5	2.563	884706	1575	2.00
6	2.600	889287	1587	1.86
		Mean=886080 %RSD=0.332		

Table-12: Method precision values of Hydrochlorthiazide by HPLC

S No.	RT(min)	Area	USP Plate count	USP tailing
1	4.488	657740	7624	1.26
2	4.468	656693	8032	1.22
3	4.398	658616	8536	1.42
4	4.416	650171	8981	1.10
5	4.410	652877	8795	1.21
6	4.481	650771	8229	1.14
		Mean=654478 %RSD=0.561		

Observation:

From the system precisions studies it is observed that all the parameters like %RSD of retention time and peak areas are within limits.

3. Intermediate precision (inter day):

Six sample solutions are prepared and injected on the next day into the HPLC system as per test procedure. The observations of Intermediate precision were given in below tables.

Table-13 : Intermediate precision values of Reserpine by HPLC

S No.	RT(min)	Area	USP Plate count	USP tailing
1	7.997	1174223	12591	1.32
2	7.966	1170958	13437	1.20
3	7.840	1174927	13470	1.35
4	7.880	1173702	8407	1.36
5	7.879	1173247	9540	1.40
6	7.993	1176831	11580	1.31
		Mean=1173981 %RSD=0.165		

Table 14 : Intermediate precision values of Dihydralazine sulphate by HPLC

S No.	RT(min)	Area	USP Plate count	USP tailing
1	2.632	856265	1422	1.96
2	2.608	852690	1404	1.93
3	2.561	855679	1610	2.00
4	2.570	852640	1699	1.89
5	2.563	857930	1612	1.93
6	2.600	845972	1669	1.76
		Mean=853529 %RSD=0.497		

Table-15:Intermediate precision values of Hydrochlorthiazide by HPLC

S No.	RT	Area	USP Plate count	USP tailing
1	4.488	657740	7624	1.26
2	4.468	656693	8032	1.22
3	4.398	653471	8476	1.21
4	4.416	660442	8849	1.27
5	4.410	652877	8795	1.21
6	4.481	650771	8229	1.14
		Mean=655332 %RSD=0.546		

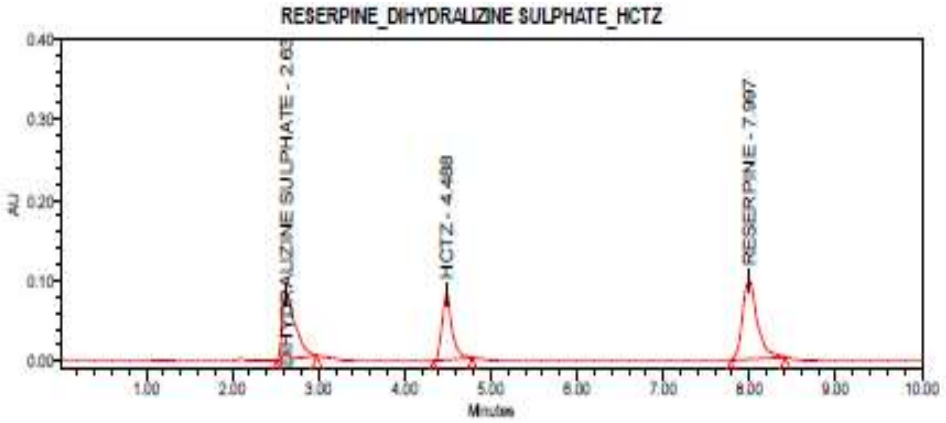


Figure8:Representative chromatogram of Intermediate precision

Observation:

From the system precisions studies it is observed that all parameters like %RSD of retention time and peak areas are within limits.

Robustness:

Robustness of the proposed analytical method is a measure of its capacity to remain unaffected, and it reflects the reliability of the analysts with respect to deliberate changes in the parameters such as flow rate, column temperature, mobile phase ratio, wave length etc.

1. **Effect of variation of organic phase**:

A study is conducted to determine the effect of variation in mobile phase ratio by changing the ratio of acetonitrile :buffer from 75:25%v/v to 70:30 %v/v and 65:35%v/v by preparing standard solution and injecting in to HPLC system. The readings of variation of organic phase were given in below tables.

Table 16 : Effect of variation of organic phase of Reserpine

S.NO	Composition (v/v)	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	75:25	7.839	1219381	10940	1.42
2	75:25	7.764	1229456	13695	1.20
%RSD			0.644		
1	70:30	7.880	1256499	8159	1.25
2	70:30	7.879	1253805	9307	1.24
%RSD			0.291		
1	65:35	6.302	1061371	9162	1.40
2	65:35	6.366	1058660	10540	1.04
%RSD			0.503		

Table -17 :Effect of variation of organic Phase of Dihydaralazine sulphate

S.NO	Composition (v/v)	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	75:25	2.551	783560	1704	1.93
2	75:25	2.507	784686	2099	1.75
%RSD			0.244		
1	70:30	2.570	884041	1640	1.97
2	70:30	2.563	884706	1575	2.00
%RSD			0.332		
1	65:35	2.205	779773	1128	2.88
2	65:35	2.019	775669	1287	1.80
%RSD			0.837		

Table 18: Effect of variation of organic Phase of Hydrochlorthiazide

S.NO	Composition (v/v)	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	75:25	4.412	674568	8873	1.34
2	75:25	4.371	674848	11361	0.98
%RSD			0.336		
1	70:30	4.416	650171	8981	1.10
2	70:30	4.410	652877	8795	1.21
%RSD			0.561		
1	65:35	3.496	628546	7080	1.69
2	65:35	3.526	628551	7945	1.26
%RSD			0.130		

Wavelength:

A study is conducted to determine the effect of variation in wave length by preparing standard solutions and injecting in to HPLC system**.**

Table-19 : Effect of wave length on Reserpine

S.NO	Wave length (nm)	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	235	8.444	203447	8455	1.24
2	235	8.281	202935	7320	1.28
%RSD			0.776		
1	240	7.997	1251130	12186	1.43
2	240	7.966	1249896	13082	1.38
%RSD			0.291		
1	245	8.445	290645	8248	1.33
2	245	8.281	287971	7093	1.39
%RSD			0.894		

Table-20: Effect of wave length on Dihydralazine sulphate

S.NO	Wave length	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	235	2.206	1953904	1247	1.39
2	235	2.208	1954734	1086	1.28
%RSD			0.047		
1	240	2.632	881948	1382	2.03
2	240	2.608	887765	1332	2.04
%RSD			0.332		
1	245	2.206	347779	1155	1.451.10
2	245	2.226	337584	1131	
%RSD			0.726		

Table- 21: Effect of wave length on Hydrochlorthiazide

S.NO	Wave length	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	235	4.779	751783	12517	1.20
2	235	4.726	760273	10707	1.24
%RSD			0.579		
1	240	4.488	657740	7624	1.26
2	240	4.468	656693	8032	1.22
%RSD			0.561		
1	245	4.779	649776	12208	1.29
2	245	4.726	639345	10589	1.26
%RSD			1.216		

Effect of variation of Flow rate:

A standard solution was prepared and injected in to the HPLC system by keeping fow rates 0.8 mL/min and 1.2 mL/min , the effect is evaluated**.**

Table-22 : Effect of variation of flow rate of Reserpine

S.NO	Flow rate(ml/min)	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	0.8	7.839	1228413	10906	1.45
2	0.8	7.764	1229456	13695	1.20
%RSD			0.066		
1	1.0	7.997	1251130	12186	1.43
2	1.0	7.996	1249896	13082	1.38
%RSD			0.291		
1	1.2	6.302	886631	10233	1.06
2	1.2	6.366	871836	12280	1.17
%RSD			1.764		

Table-23 : Effect of variation of flow rate of Dihydralazine sulphate

S.NO	Flow rate (mL/min)	Retention time(min)	Peak area	Theoretical plates	Tailing factor
1	0.8	2.551	817648	1661	2.04
2	0.8	2.507	819318	1987	1.84
%RSD			0.540		
1	1.0	2.632	881948	1382	2.03
2	1.0	2.608	887765	1332	2.04
%RSD			0.332		
1	1.2	2.205	669349	1247	1.90
2	1.2	2.019	660611	1459	1.91
%RSD			0.721		

Table-24 : Effect of variation of flow rate of Hydrochlorthiazide

S.No	Flow rate(ml/min)	Retention time (min)	Peak area	Theoretical plates	Tailing factor
1	0.8	4.412	719430	8721	1.53
2	0.8	4.371	723822	10516	1.17
%RSD			0.344		
1	1.0	4.488	657740	7624	1.26
2	1.0	4.468	656693	8032	1.22
%RSD			0.561		
1	1.2	3.496	511345	7805	1.20
2	1.2	3.526	512276	9430	1.16
%RSD			0.524		

Limit of detection(LOD)& Limit of quantitation(LOQ):

LOD & LOQ were calculated on the peak area using the following equations**.**

LOD : 3.3α/S
LOQ : 10 α/S

Table-25 :Limit of detection and limit of quantitation

S.NO	Sample	LOD	LOQ
1	Reserpine	0.047	0.144
2	Dihydralazine sulphate	0.0305	0.10179
3	Hydrochlorothiazide	0.065	0.219

Stability studies:

The solution stability of Reserpine ,Dihydralazine sulphate, and Hydrochlorothiazide diluents were determined by storing sample solutions in a tightly closed volumetric flask at room temperaturefor 24 hrs. The amount of Reserpine, Dihydralazine sulphate, and Hydrochlorothiazide were measured at different time intervals like intial, 12 and 24hrs and results obtained were compared with freshly prepared Reserpine, Dihydralazine sulphate, Hydrochlorothiazide solutions.

Table 26 : Solution Stability studies for Reserpine

Stability	Sample weight(mg)	Area counts	%label claim	%deviation
Intial	685.5	258087	100.1	0.0
12hrs	675.5	250632	100.2	-0.4
24hrs	680.5	248508	100.0	0.6

Table 27: Solution stability studies for Dihydralazine sulphate

Stability	Sample weight(mg)	Area counts	%label claim	%deviation
Intial	662.8	888258	100.5	0.00
12hrs	654.4	875345	100.3	0.1
24hrs	656.6	876517	100.1	0.4

Table-28: solution stability of Hydrochlorthiazide

Stability	Sample weight(mg)	Area counts	%label claim	%deviation
Intial	685.5	712924	99.9	0.00
12hrs	675.5	705325	100.3	-0.5
24hrs	680.5	707856	99.9	0.5

Forced degradation studies:

Regulatory guidance in ICH Q2A, Q2B, Q3B and FDA 21 CFR section 211 requires the development and validation of stability indicating potency assays.

Preparation of working standard solution :

About 0.1 mg of Reserpine, 10 mg of Dihydralazine sulphate and 10mg of Hydrochlorothiazide pure drugs were accurately weighed and transferred to 50 ml volumetric flask and made up to the mark with diluent**.**

Table 29: Results of forced degradation studies of Reserpine

S.NO	Sress conditions	R_t(min)	Peak area	Plate count	USP tailing	% degraded	Purity angle	Purity threshold
1	Control	8.444	261022	8230	1.33	-0.4	1.149	8.48
2	Acid	8.444	207899	9619	1.23	21.6	0.604	8.191
3	Alkali	8.407	220875	9323	1.23	21.1	0.618	8.066
4	Peroxide	8.444	216716	9295	1.26	21.9	0.646	8.207
5	Reduction	8.199	206614	9878	1.23	24.1	3.061	10.359
6	Thermal	8.338	172898	8617	0.92	23.3	1.349	9.714
7	Photo	8.407	216904	9449	1.20	21.2	0.609	8.058
8	Humidity	8.199	207094	9772	1.23	24.8	3.163	10.368
9	Hydrolysis	8.389	210405	7411	1.19	24.1	1.294	9.635
10	Heat	8.407	223792	9246	1.16	25.6	0.612	8.053

Table 30: Results of Forced degradation of Dihydralazine sulphate

S. N0	Stress conditions	Rt(min)	Peak area	Plate count	USP Tailing	% degraded	Purity angle	Purity Threshold
1	CONTROL	2.206	893881	1219	1.42	-0.2	1.335	7.195
2	ACID	2.206	843886	1256	1.41	20.7	1.287	7.153
3	ALKALI	2.235	889208	1243	1.44	21.1	0.941	7.08
4	PEROXIDE	2.206	804869	1295	1.44	28.8	1.266	7.148
5	REDUCTION	2.206	840477	1457	1.26	25.6	1.504	7.376
6	THERMAL	2.195	796963	1134	1.25	23.7	0.596	7.335
7	PHOTO	2.235	782835	1383	1.32	23.1	0.828	7.074
8	HUMIDITY	2.206	848579	1447	1.29	20.2	1.517	7.383
9	HYDROLYSIS	2.195	836535	1099	1.24	20.3	0.595	7.358
10	HEAT	2.235	899523	1234	1.43	20.4	0.942	7.081

Table 31: Results of Forced degradation of Hydrochlorothiazide

S. N 0	Stress conditions	Rt(min)	Peak area	Plate count	USP Tailin g	% degraded	Purity angle	Purity Threshold
1	CONTROL	4.779	719090	12218	1.29	-0.4	0.122	7.194
2	ACID	4.779	687746	12467	1.23	20.1	0.073	7.13
3	ALKALI	4.768	670405	12992	1.27	20.9	0.085	7.123
4	PEROXIDE	4.779	701995	12332	1.25	22.1	0.082	7.14
5	REDUCTION	4.703	645133	12995	1.23	24.1	0.081	7.191
6	THERMAL	4.765	584310	11435	1.08	25.6	0.083	7.204
7	PHOTO	4.768	666998	13025	1.25	21.7	0.084	7.119
8	HUMIDITY	4.703	676315	12590	1.25	21.4	0.091	7.205
9	HYDROLYSIS	4.765	678971	10314	1.19	20.2	0.122	7.245
1 0	HEAT	4.768	694992	12671	1.23	20.5	0.086	7.123

Results & Discusions :

Table 32: System suitability parameters:

Parameter	Acceptance criteria	Observed values for Reserpine	Observed values for Dihydralazine sulphate	Observed values for Hydrochlorothiazide
Linearity range Correlation coefficient	Correlation coefficient r^2>0.999	r^2=0.999	r^2=0.999	r^2=0.999
System precision	RSD<2%	%RSD=0.603	%RSD=0.311	%RSD=0.325
Intermediate precision	RSD<2%	%RSD=0.165	%RSD=0.497	%RSD=0546
Method precision	RSD<2%	%RSD=0.291	%RSD=0.332	%RSD=0.561
Accuracy	Recovery 98-102%(individual)	%recovery =100.3	%recovery=100.6	%recovery =100.2
Solution stability	>12hrs	Stable up to 24hrs	Stable up to 24 hrs %RSD=0.325	Stable up to24hrs

		%RSD =0.646		%RSD=0.271
Robustness	RSD NMT 2% in modified condition	Complies	Complies	Complies
	Flow minus	%RSD=0.066	%RSD=0.540	%RSD=0.344
	Flow plus	%RSD=1.764	%RSD=0.721	%RSD=0.524
	Organic plus	%RSD=0.503	%RSD=0.837	%RSD=0.130
	Organic minus	%RSD=0.644	%RSD=0.1244	%RSD=0.336
	Wavelength plus	%RSD=0.894	%RSD=1.726	%RSD=1.216
	Wavelength minus	%RSD=0.776	%RSD=0.047	%RSD=0.579

Conclusion:

In the present investigation new analytical method has been developed for the simultaneous estimation of potent drugs Reserpine, Dihydralazine sulphate, Hydrochlorothiazide . There is no analytical method available to determine the same combination of drugs. So we have selected and developed a new analytical method for the routine analysis of Reserpine, Dihydralazine sulphate and Hydrochlorothiazide in bulk and combined dosage forms according to ICH guidelines.

Acknowledgement:

I express my sincere thanks to Dr.k.Santhakumari, Dr.SK. Abdul Rahaman and Managment of Nirmala college of pharmacy for encouragement throughout my research work. And also thankful to ICON labs Vijayawada for providing the Reserpine, Dihydralazine sulphate, Hydrochlorothiazide pure drugs.

References :

1. https://en.wikipedia.org/wiki/Combination_therapy
2. https://www.ndrugs.com/?s=reserpine/dihydralazine%20sulfate/hydrochlorothiazide.
3. https://www.belmarrahealth.com › Heart Health.
4. https://en.wikipedia.org/wiki/Hydrochlorothiazide.
5. Indolealkaloids..Major Types Of Chemical Compounds In Plants & Animals Part II: Phenolic Compounds, Glycosides & Alkaloids. Wayne's Word: An On-Line Textbook of Natural History. 2005.
6. https://www.sigmaaldrich.com/catalog/product/sigma/d2697?lang=en&region
7. H.H.Willard,L.L.Merritt, J.A.Dean,F.A.Settle. Instrumental methods of analysis, 7th Edition, New delhi: CBS Publishers; 1986, pg no:2-3,580-613.
8. Phyllis R.Brown, Kingston, Rhode Island, HPLC and CE-principles and practice, pg no:24-31
9. Sharma B.K. Chromatography. 23rded. Meerut :Goel publishing house, 2004, pg no: 288-302
10. James Swabrik. Encyclopedia of pharmaceutical technology, 3rdedition, vol-I. 2007,pg no :526-537.
11. Sethi . P.D, HPLC , Quantitative analysis of pharmaceutical formulations . Isted, vol-I. New Delhi : CBS publishers and distributors ;2001. Pgno :3-72, 94-95, 116-120.
12. Snyder L.R, Kirkland JJ, Glajch JL. Practical HPLC method development. 2nd ed. Wiley inter science publication; 1997, pg no: 1-13, 687-705.
13. Indian pharmacopoeia , vol-3, pharmacopoeia commission, Ministry of Health &family welfare, Ghaziabad, India, 6th Edition, 2010.
14. Napa. Delhiraj*, Sockalingam. Anbazhagan validated liquid chromatographic method for the estimation of anti hypertensive mixture in pharmaceutical dosage forms world journal of pharmacy and pharmaceytical sciences volume 3, Issue 6, 1916-1927.
15. Sarojkumarraul*,B.V.V Ravi kumar, Ajay kumarPattnaik, Nagi Reddy Neelakantarao. RP-HPLC method development and validation for the estimation of Dihydralazine in bulk and pharmaceutical dosage forms.Int j pharm 2013; 3 (1): 116-121.
16. Anandarao Bonthala*, and T.A.D. Surya kumar. Development and validation of RP-HPLC method for the estimation of Hydrochlorothiazide and Candesartan cilexetil in pharmaceutical dosage form. Int j pharm 2013;3(1) :166-169.

17. Mukhopadhyay S* , Kadam K, Sawat L, Nachane D, Pandita N, simultaneous determination of related substances of Telmisartan and Hydrochlorothiazide in tablet dosage form by using RP-HPLC method . J pharmaBioallied sci. 2011jul; 3(3) :375-83.
18. Hfez HM*,Elshanawane AA, Abdelaziz LM, Kamal MM, quantitative determination of three angiotensin –II receptor antagonist in the presence of Hydrochlorothiazide by RP-HPLC in their tablet preparatios. Iran J pharma Res.2013.
19. Meyyanathan SN[1], Rajan S, Muralidharan S, Birajdhar AS, B A validated RP-HPLC Method for simultaneous estimation of Nebivolol and Hydrochlorthiazide in tablets. Indian J pharm sci.- 2008 sep, 70 (5): 687-9.
20. Jain PS[1], Patel MK, Gorle AP, Chaudhari AJ, Surana SJ stability – indicating method for simultaneous estimation of olmisartanmedoxamil, amlodipine besylate and hydrochlorthiazide by RP-HPLC in tablet dosage form. J Chromatography sci. 2012 sep ; 50 (8): 680-7.
21. Patel HU[1], Suhagia BN, Patel CN. Simultaneous analysis of Eposartan and hydrochlorthiazide in tablets by HPLC.Pharm methods.2011 Apr; 2(2)143-7.
22. Dr.Ravisankar , Text book of pharmaceutical analysis, 2010; 4th edition:1-18.
23. Skoog , D.A., Holler, F,J. and nieman, T.A.:principles of instrumental analysis, 2004; 4th edition: 724.
24. David G. Watson, pharmaceutical analysis, 2005; 3rd EDITION;268.
25. ICH Guidelines, Q2R1 Validation of analytical procedures ; Text and methodology , ICH, Geniva , Switzerland, 2005.
26. http://www. Webmed.com/drugs/2/drug-148989/levocetrizine-oral/details.
27. http://en.wikipedia.org/wiki/reserpine.
28. http://www.drugbank.ca/drugs/DB00388.
29. http://www..drugs.com/ingredient/dihydralazine sulphate.html
30. https://www.ndrugs.com/?s=reserpine/dihydralazine%20sulfate/hydrochlorothiazide

*****