RESEARCH ARTICLE

Development and Validation of RP-HPLC method for simultaneous estimation of Methyldopa and Hydrochlorothiazide in Pharmaceutical Dosage Form

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ABSTRACT

A new simple accurate and economical reverse phase high performance liquid chromatographic method was developed for the determination of Methyldopa and Hydrochlorothiazide in bulk and tablet dosage form. The separation was eluted on a Hypersil BDS C_8 column (250 mm x 4.6 mm; 5µ) using a mobile phase mixture of mixed phosphate buffer 5.5 and acetonitrile in a ratio of 50:50 v/v at a flow rate of 1.0ml/min. The detection was made at 287 nm. The retention times were 2.17min for Methyldopa and 3.56min for Hydrochlorothiazide. Calibration curve was linear over the concentration range of 62.5-375.0 µg/ml for Methyldopa and 6.25 to 37.5 µg/ml for Hydrochlorothiazide. The propose method was validated as per the ICH guidelines parameters like Linearity, specificity, precision, accuracy, robustness and ruggedness. The method was accurate, precise, specific and rapid found to be suitable for the quantitative analysis of the drug and dosage form.

Keywords: Method development and validation, Methyldopa, Tablets, C_8 column, RP-HPLC.

INTORDUCTION

Methyldopa is (2S)-2-amino-3-(3, 4-dihydroxyphenyl)-2-methylpropanoic acid (figure1a). Hydrochlorothiazide is 6-chloro-1, 1-dioxo-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide (figure1b). Methyldopa and Hydrochlorothiazide are official in Indian Pharmacopoeia and U.S. Pharmacopoeia, but there is no official method for the combination. Both drugs in combination of tablet dosage form in the ratio of 250:25 mg Methyldopa and Hydrochlorothiazide respectively. The formulation of Methyldopa and Hydrochlorothiazide is available with trade name Aldoril-25, the medication is used to treat high blood pressure (hypertension). Lowering high blood pressure helps in preventing strokes, heart attacks and kidney problems. Methyldopa works by relaxing blood vessels so blood can flow more easily. Hydrochlorothiazide is a "water pill" (diuretic), it increases the urine output and helps to relax the blood vessels so that blood can flow more easily. A few methods have been reported including response surface methodology [3], HPLC [4, 5]. With this present proposed method both Methyldopa and Hydrochlorothiazide estimates simple and economical in tablet formulation.

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EXPERIMENTAL

2.1 Chromatographic Conditions

Waters e 2695 separation module with high pressure liquid chromatographic instrument provided with a Hypersil BDS C_8 column (250 mm x 4.6 mm ; 5 μ) and 2489 UV-Visible detector, auto injector, auto sampler with Empower 2 software from Waters corporation, Milford USA was employed in the study.

2.2 Chemicals and Reagents

HPLC grade acetonitrile, and Potassium dihydrogen phosphate AR Grade, Potassium Hydroxide AR grade were purchased from SD Fine Chem Mumbai, India were used in the study, reference samples were obtained from M/s. Bio-Leo Analytical Labs India Pvt Ltd, Hyderabad, India, and the formulation samples were purchased from local market.

2.3 Preparation of Mobile phase

Mix an accurately weighed 1.36g of potassium dihydrogen phosphate in 1000ml of water adjust pH 5.5±0.1 with dilute potassium hydroxide and acetonitrile in the ratio 50:50 v/v was filtered through 0.22µ membrane filter and was degassed. Mobile phase was used as diluent for preparing the working solution of the drug. The mobile phase was filtered and sonicated by using Bio-Technics India, Mumbai before use. The flow rate of the mobile phase was maintained at 1ml/min. The column temperature was maintained at 30ºC and the detection of the drug was carried out at 287nm.

2.4 Preparation of stock and working standard solution of Methyldopa and Hydrochlorothiazide

About 250.0 mg of Methyldopa and 25.0 mg of Hydrochlorothiazide was weighed accurately on Sartorius semi micro balance model-CPA225D and transfers in to 100ml volumetric flask the solution was sonicated and the resulting solution was diluted with the mobile phase to get a working standard solution of 2500 µg/ml Methyldopa and 250 µg/ml Hydrochlorothiazide. Further dilute 10 ml to 100 ml with mobile phase gives 250 µg/ml Methyldopa and 25 µg/ml Hydrochlorothiazide.

2.5 Sample Preparation

Weighed accurately previously weighed and crushed 20 tablets powder equivalent 1 tablet weight which contains 250mg of Methyldopa and 25mg of Hydrochlorothiazide transferred to 100ml volumetric flask make up to the mark with mobile phase sonicated and filtered through whatman filter paper. Further dilute 10 ml to 100 ml with mobile phase.

METHOD VALIDATION

The method was validated the parameters like system suitability, linearity, precision, intermediate precision, accuracy, robustness, Limit of detection (LOD), limit of quantitation (LOQ).

System suitability was evaluated by injecting six times the working standard solution into the HPLC. The parameters like retention time, peak area, tailing factor, resolution were measured.

Linearity and the detector response for Methyldopa and Hydrochlorothiazide was demonstrated by prepared solutions over the range 25% to 150% level of standard solution injected 20µl into HPLC record the areas of the concentrations.

The precision was evaluated six sample preparations representing a single batch were prepared and injected, the %RSD for both retention time and area were determined.

The ruggedness of the method was injected six preparations of a single batch sample by different analyst, different column and different instrument, the %RSD for both retention time and area were determined.

The Accuracy of the test method was prepared recovery samples ie., test sample with known quantities at the level of 50%, 100% and 150% concentration.

The robustness of the test method was injected the test preparation under normal condition(ie.,as such condition) and of the altered conditions column temperature 30±5°C, flow rate 1±0.1ml, buffer pH 5.5±0.2, the initial and all changed system suitability parameters were studied.

The Limit of detection and quantitation were calculated by using 3.3 multiplied standard deviation divided by slope and 10 multiplied standard deviation divided by slope respectively.

RESULTS AND DISCUSSION

The present study was aimed at developing a simple precise and accurate HPLC method for the analysis of Methyldopa and Hydrochlorothiazide in bulk drug and in pharmaceutical dosage form. In order to achieve optimum separation of the component peaks, mixture of acetonitrile with water in different combinations were tested as mobile phase on a C_8 stationary phase. A mixture of Phosphate buffer pH 5.5: acetonitrile in a proportion of 50:50 v/v was selected as the chromatographic peaks were well defined and resolved with no tailing, The retention time obtained for Methyldopa was 2.17±0.1 min and for

Hydrochlorothiazide was 3.56±0.1min, the optical and system suitability parameters are tabulated in Table 1 and the system suitability chromatogram shown in figure2.

Linearity of the peak area response was determined by taking measurement at Six concentration prints (6 replicates at each point) working standard dilution of Methyldopa and Hydrochlorothiazide in the range of 62.5-375.0µg/ml and 6.25 to 37.5 µg/ml respectively. 20µl quantity of the dilution was injected each time in to the column. The drug elutes was monitored at 287 nm at a column temperature of 30ºC and the corresponding chromatograms were obtained. Form these chromatograms the mean peak areas were calculated and a plot of concentration over the peak area was constructed. Each of the samples was injected Six times and the Sample retention times were observed in all cases. The peak areas of Methyldopa and Hydrochlorothiazide were reproducible as indicated by low coefficient of variation. A good linear relationship (r^2 = 1.0) was observed for both Methyldopa and Hydrochlorothiazide, The linearity of six different concentrations of overlaid chromatogram of Methyldopa and Hydrochlorothiazide shown in figure 3. The regression concentration and areas are given in table 2.

The precision of test method was determined and the percentage of relative standard deviation was found less than 1.0 for both retention time and area, indicating the method is precise and the results were presented in Table 3, a representative overlaid chromatogram shown in figure 4.

The ruggedness of the method was evaluated by different analyst(analyst-2), different column(column-2) and different instrument(instrument-2), the % RSD was less than 1.0, consider precision results for analyst-1, column-1 and instrument-1, the mean %RSD for both precision and intermediate precision less than 1.0 shows the method is rugged and the results were tabulated in Table 3.

The accuracy of the test method was evaluated and the recovery results were found between 98 to 102% indicates the method is accurate for the estimation of Methyldopa and Hydrochlorothiazide different dosage forms and the results were tabulated in Table 4.

The deliberate changes in the method have not much affected the system suitability parameters like peak tailing, Theoretical plates and resolution. The standard solution of the drug was stable up to 24 hrs. This indicated the robustness of the method. The robustness study results were tabulated in table 5 a representative overlaid chromatogram shown in figure 5.

The diluted preparations of marketed tablets were injected in duplicate and the results were calculated and presented in Table 6. The sample preparation chromatogram shown in figure 6. The absence of additional peaks indicated non-interference of common excipients used in the tablets.

Hence it can be concluded that the proposed HPLC method is evident very fast and economical for estimation of Methyldopa and Hydrochlorothiazide in pharmaceutical dosage forms.

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Table 1: Optical and System suitability parameters

Parameter	Methyldopa	Hydrochlorothiazide
Concentration(µg/ml)	62.5-375	6.25-37.5
Slope(m)	6972	25594
Correlation Coefficient(r^2)	1.0	1.0
Intercept(b)	-273.5	-1466
USP Tailing	1.14	1.32
Theoretical Plates	2019.92	2962.64
Resolution	---	5.27
LOD(µg/ml)	5.7565	0.3750
LOQ(µg/ml)	17.4439	1.1365

Table 2: Calibration data of the proposed method

Methyldopa Conc(mcg/ml)	Mean Area	Hydrochlorothiazide Conc(mcg/ml)	Mean Area
62.500	432812	6.25000	156474
125.000	873462	12.50000	319253
187.500	1308748	18.75000	476636
250.000	1743813	25.00000	639438
312.500	2174451	31.25000	799694
375.000	2615983	37.50000	957431

Table 3: Precision, method precision results

PRECISION					METHOD PRECISION			
Methyldopa			Hydrochlorothiazide		Methyldopa		Hydrochlorothiazide	
S. No.	**RT**	**AREA**	**RT**	**AREA**	**RT**	**AREA**	**RT**	**AREA**
1	2.281	379787	3.762	1363665	2.284	381237	3.765	1364023
2	2.281	380108	3.763	1363958	2.282	380045	3.763	1361543
3	2.281	377369	3.761	1356776	2.285	380343	3.766	1362655
4	2.283	375783	3.763	1347133	2.284	385409	3.766	1366567
5	2.284	378786	3.766	1357368	2.281	382680	3.762	1364360
6	2.286	378740	3.769	1358723	2.283	384890	3.766	1365680
Avg	2.283	378428.833	3.764	1357937	2.283	382434	3.765	1364138
Stdev	0.00206	1613.21361	0.002966	6133.161	0.00147	2300.24	0.001751	1858.689
%RSD	**0.09**	**0.43**	**0.08**	**0.45**	**0.06**	**0.60**	**0.05**	**0.14**

Table 4: Accuracy data (Triplicate values at 50,100 &150 percent levels)

Parameter	Initial Amount(µg)	Amount added(µg)	Amount Recovered(µg)	% of recovery	Mean % of Recovery
Methyldopa					
50% level	125	187.5	187.18	99.83	98.83
100%level	125	250	249.45	99.78	99.78
150%level	125	312.5	311.91	99.81	99.81
Hydrochlorothiazide					
50% level	12.5	18.75	18.65	99.48	99.48
100%level	12.5	25	24.915	99.66	99.66
150%level	12.5	31.25	31.04	99.33	99.33

Table 5: Robustness Study

Drug Name	Parameter	Chromatographic Conditions			
		Retention time	Area	Theoretical Plates	Tailing Factor
Methyldopa	Buffer Change ±2%				
	45%v/v	2.032	1687976	2213.32	1.11
	50%v/v	2.170	1744299	2019.92	1.14
	55%v/v	2.402	1846589	2014.56	1.16
	Flow Change±0.1				
	0.9ml/min	2.052	1897291	2181.73	1.15
	1.0ml/min	2.170	1744299	2019.92	1.14
	1.1ml/min	2.029	1638662	2889.61	1.13
Hydrochlorothiazide	Buffer Change ±2%				
	45%v/v	3.088	615648	2986.45	1.30
	50%v/v	3.563	636989	2962.64	1.32
	55%v/v	4.081	665849	2768.52	1.35
	Flow Change±0.2				
	0.9ml/min	3.868	692256	2072.76	1.34
	1.0ml/min	3.563	636989	2962.64	1.32
	1.2ml/min	3.325	600880	2875.05	1.12

Table 6: Assay Results

Drug	Amount present/tablet	% of Assay
Methyldopa	248.7 mg	99.48
Hydrochlorothiazide	24.9425 mg	99.77

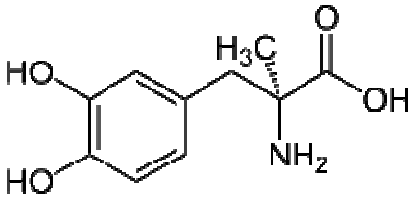


Fig 1a: Structure of Methyldopa

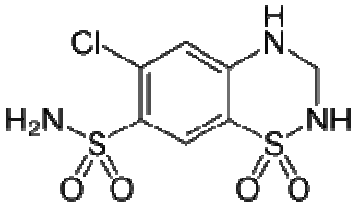
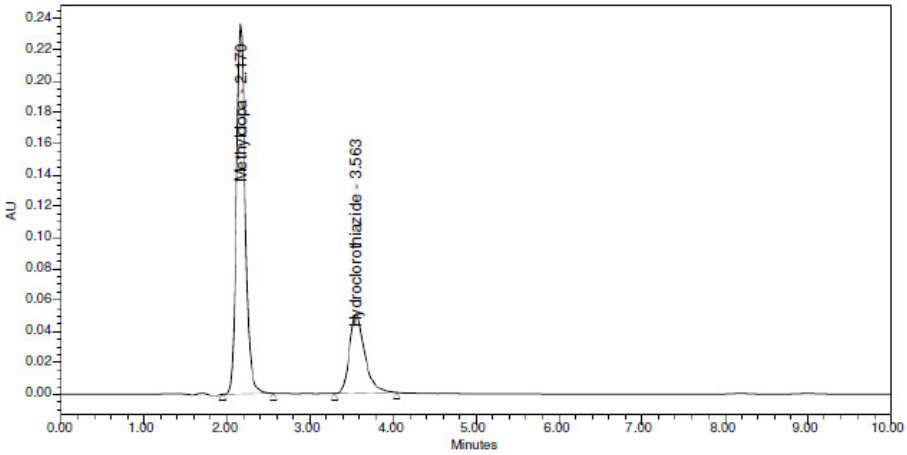


Fig 1b: Structure of Hydrochlorothiazide



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Methyldopa	2.170	1744299	235490	73.250		1.14	2019.92
2	Hydroclorothiazide	3.563	636989	50697	26.750	5.27	1.32	2962.64

Figure 2: System suitability chromatogram

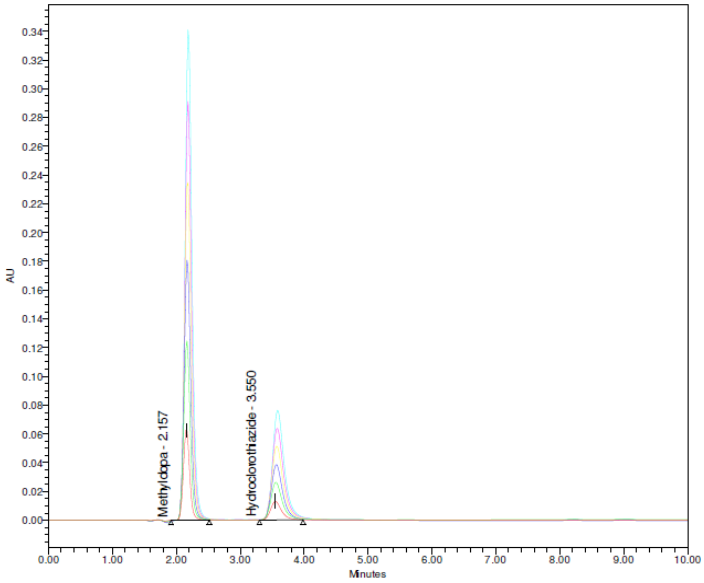


Fig 3: Methyldopa and Hydrochlorothiazide Overlaid linearity chromatogram

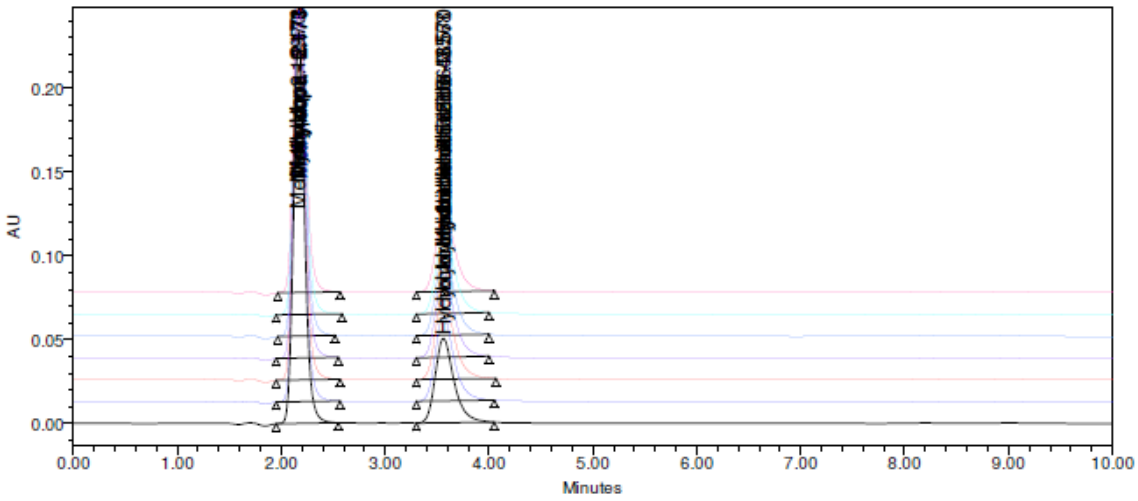


Fig 4: Methyldopa and Hydrochlorothiazide Overlaid Precision chromatogram

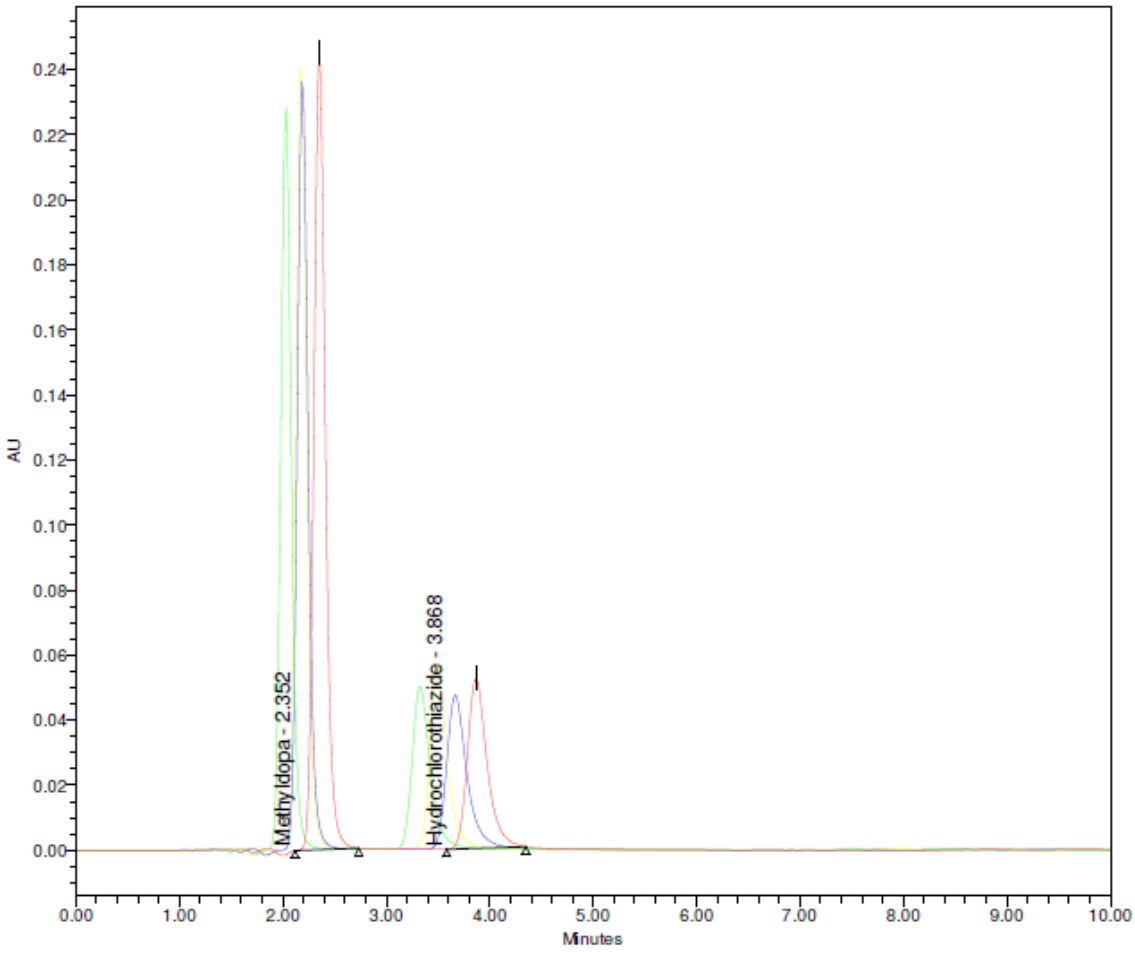
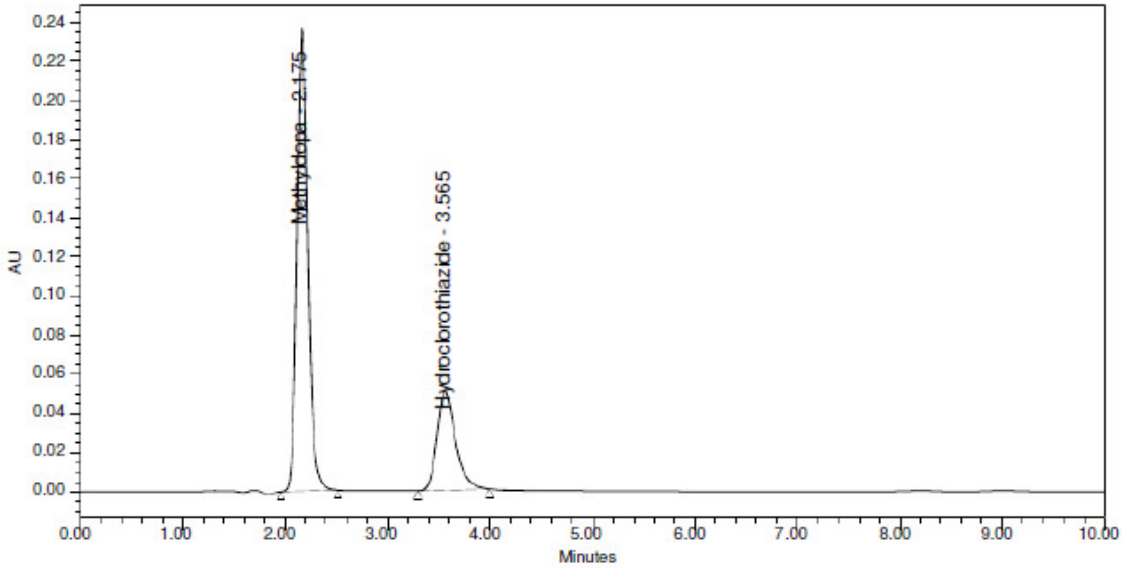


Fig 5: Methyldopa and Hydrochlorothiazide Overlaid robustness chromatogram



	Peak Name	RT	Area	Height	% Area	USP Resolution	USP Tailing	USP Plate Count
1	Methyldopa	2.175	1750246	237352	73.50		1.11	2066
2	Hydroclorothiazide	3.565	640213	51050	26.50	5.30	1.12	2968

Fig. 6: Methyldopa and Hydrochlorothiazide sample chromatogram

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