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Simultaneous spectrophotometric determination of methyldopa and hydrochlorothiazide in pharmaceutical dosage form by AUC and first order derivative method

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ABSTRACT

A New, Simple, Accurate And Sensitive UV-Spectrophotometric Method has been developed for simultaneous determination of Methyldopa And Hydrochlorothiazide(HCTZ) in bulk And combined dosage form .Method A is AUC method, which involved measurement of area between 276-286nm and 266-276nm for the estimation of MD and HCTZ respectively. Method B Applied first order derivative Spectrophotometry, which involved measuring the absorbance values at 271.40nm and 251.20nm of first derivative spectrum. Beer's law obeyed in concentration range of 10-60µg/ml and 2-14µg/ml for MD & HCTZ respectively by both Methods. Results of analysis were statistically reported & were found to be satisfactory.

Keywords: Methyldopa, Hydrochlorothiazide, Spectroscopy, AUC Method, First order derivative method.

INTRODUCTION

Methyldopa [3, 4] (MD) (Fig 1)is 3-(3, 4dihydrophenyl)-2-Methyl-L-alanine sequihydrate is Chemical name of methyldopa. It isWhite to yellowish white, Fine powder which may contain friable lumps it is slightly soluble in water, very slightly soluble in Ethanol (95%), practically insoluble in chloroform and in ether. It is freely soluble in dilute hydrochloric acid.

Hydrochlorothiazide [5, 6] (HCTZ)(Fig2) is 6-chloro-3, 4dihydro-2H-1, 2, 4, benzathiadiazine-7-suiphonamide. It is White or almost white, crystalline powder, odorless. Soluble in acetone, sparingly soluble in ethanol (95%).Very slightly soluble in water, it dissolves in dilute solution of alkali hydroxides Literature survey revealed UV-Visible spectrophotometric methods such as simultaneous equation method [7], Dual Wavelength method [8] and RP-HPLC [9, 10] for

the estimation of MD and HCTZ alone or in combination with other drugs. No method has been reported for this combination using Distilled water and by Area under Curve Method and derivative method. The present work therefore emphasizes on the quantitative estimation of MD and HCTZ in bulk and in their combined dosage form by UV spectroscopy. Tablet Aldorilcontain Methyldopa (250mg) and Hydrochlorothiazide (25mg) were analyzed for assay study.

MATERIALS& METHODS

Instrument [11]

The Present Work Was Carried out on Shimadzu UV 1800 Double Beam Visible Spectrophotometer Wave length range 190-1100Band Width 2nmm, with a pair of 1 cm matched quartz cells.

Reagent and Chemicals

Pharmaceutically pure sample of MD & HCTZ obtain form Flamingo Private Ltd., Nanded & Ajanta pharma. Chitegaon. Tablet Aldorilcontain Methyldopa (250mg) and Hydrochlorothiazide (25mg) purchase from local market.

Preparation of Stock Solution

Accurately Weighted 10 mg of Methyldopa & hydrochlorothiazide Propionate was transferred in 100 ml volumetric Flask dissolved separately Distilled water that give final concentration of 100 µg/ml both the drugs.

Preparation of working solution

Appropriate volume 1ml of standard stock solution of Methyldopa & Hydrochlorothiazide was transferred into 10ml volumetric flask, diluted to mark with distilled water to give concentration of 10ug/ml of each drug. The resulting solution was scanned in UV range (200nm-400nm). In spectrum Methyldopa & Hydrochlorothiazide showed absorbance maximum at 271.40 & 251.20nm respectively for derivative method & area was recorded in range 276-286nm for MD & 266-276nm for HCTZ.

Selection of analytical wavelength

Appropriate dilutions were prepared for drugs form the standard stock solution and scanned in the spectrum mode from 400 nm to 200 nm and Methyldopa & Hydrochlorothiazide Propionate showed absorbance maxima at 271.40nm & 251.20 respectively.

Preparation of Calibration curve

Appropriate volume of aliquots from standard stock solution of methyldopa and Hydrochlorothiazide were transferred to different calibrated volumetric flask of 10ml capacity. The volume was adjusted to the mark with distilled water into obtain concentration of 10,20,30,40,50&60µg/ml for MD and 2,4,6,8,10,12&14 µg/ml for HCTZ. Absorbance spectra of each solution against distilled water as blank were measured at 279.80 & 271.40nm and area was recorded in range 276-286nm & 266-276nm for MD & HCTZ respectively.

Method A: Area under Curve Method

This method is applicable when there is no sharp peak or broad spectra are obtained. It involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths λ1 and λ2. Area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. This wavelength range is selected on the basis of repeated observations so as to get the linearity between AUC and concentration. For the selection of analytical wavelength suitable dilutions of Methyldopa (10-60 µg/ml) and Hydrochlorothiazide (2-14 µg/ml) of the standard stock solutions (100 µg/ml) of both were prepared separately and scanned in the range of 200-400 nm. For Area under Curve method, the sampling wavelength ranges selected for estimation of Methyldopa and Hydrochlorothiazide are 276-286 nm (λ1-λ2) and 266-276 nm (λ3-λ4) respectively.

Method B: First Order derivative Method

The standard solutions of both the drugs were scanned in the spectrum mode from 200-400 nm. These spectrums were converted to second order derivative spectra by using derivative mode in UV probe software 2.0. The absorbance spectra, thus obtained were derivatized to remove the interference of absorbing species. The two wavelengths selected should be such that at each wavelength the absorbance difference between the components

should be as large as possible. From the examination of the second derivative spectra of Methyldopa and Hydrochlorothiazide, 271.40 nm (λ1) and 251.20 nm (λ2) were selected as working wavelengths for the second order derivative spectroscopy.

Analysis of Tablet Dosage form

Twenty Tablet were Weighed and ground to fine powder. An accurately Weighed quantity equivalent to 250 of MD & 25 mg of HCTZ was weighed and transferred to 100 ml volumetric flask Containing water sonicated for 20 min and volume was made uptothe mark with the same solvent and filtered through Whatmann filter paper (no.41). Aliquot portion (0.25 containing, 25µg/ml of MD and 0.025 Containing 2.5µg/ml of HCTZ) were transferred to 10 ml volumetric flask and volume was adjusted to mark with distilled water and the area was recorded in the range 276nm-286nm & 266nm-276nm.Andabsorbance was recorded in the wavelength 271.40nm& 251.20nm.

VALIDATION OF METHODS [12, 13]

Linearity

Methyldopa in the formulation was found to be linear in the concentration range of 10-60µg/ml while Hydrochlorothaizide 2-14 µg/ml at 276-286 nm and 266-276 nm and reported in Table.no.9.Methyldopa in the formulation was found to be linear in the concentration range of 10-60µg/ml while Hydrochlrothaizide 2-14 µg/ml at 271.40 nm and 251.20 nm and reported in Fig .3 and 4.

Precision

Precision of an analytical method is the degree of agreement among individual test results. Precision of the method was verified by using stock solutions in the ratio of 1:2 containing 25 µg/m MD and 2.5 µg/ml of HCTZ. System repeatability was done by repeating the assay three time of the same concentration after every two hours on the same day for intraday precision. Interday precision was carried out by performing the assay sample sets after 24 hours and 48 hours.

Accuracy

Accuracy of an analytical method is the closeness of the test result obtained by that of the true value. It was ascertained on the basis of recovery studies performed at different levels (80%, 100%, 120 %) of concentrations.

Ruggedness

Ruggedness of the proposed method is determined by analysis of aliquots from homogenous slot by two analyst using same operational and environmental conditions.

Sensitivity

Sensitivity of the proposed method was estimated in terms of Limit of Detection (LOD) and Limit of Quantitation (LOQ). LOD = 3.3 SD/S and LOQ = 10 SD/S, where SD is the residual standard deviation and S is the slope of the line. LOD was found to be 0.61829 µg/ml for MD & 0.819 µg/ml LOQ was found to be 1.07636 µg/ml for MD 1.092 µg/ml for HCTZ.

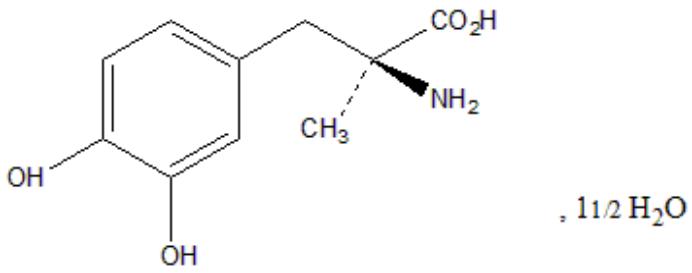

Fig 1. Chemical Structure of Methyldopa

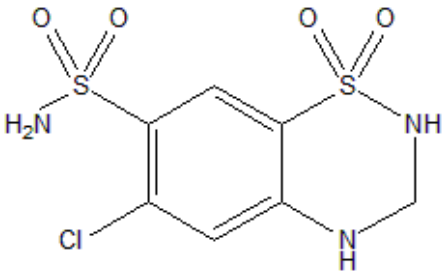

Fig. 2: Chemical Structure of Hydrochlorothiazide

Table 1: Optical characteristics and other Parameter Method A

Parameter	Methyldopa	Hydrochlorothiazide
λ max (nm)	279.80nm	271.40nm
Beer's law limits (µg / ml)	10-60 µg/ml	2-10µg/ml
Regression equation (Y*)	Y=0.011x + 0.005	Y = 0.0055x+ 0.003Y
Slope (b)	0.011	0.055
Intercept (a)	0.005	0.003
Correlation coefficient (r2)	0.999	0.999
Accuracy(%RSD) 80		
100		
120		
Precision (%RSD) Intraday	0.153161	0.436326
Interday	0.40298	0.60938
LOD	0.66	0.36
LOQ	2	1.09

Table 2: Optical characteristics and other Parameter Method B

Parameter	Methyldopa	Hydrochlorothiazide
λ max (nm)	271.40nm	251.20nm
Beer's law limits (µg / ml)	10-60 µg/ml	2-10µg/ml
Regression equation (Y*)	Y = 0.0007 x + 0.0005	Y= 0.00161x + 0.00008
Slope (b)	0.0007	0.00161
Intercept (a)	0.0005	0.00008
Correlation coefficient (r2)	0.999	0.998
Accuracy(%RSD)		
80		
100		
120		
Precision (%RSD) Intraday	0.64506	0.51504
Interday	0.2012	0.6053
LOD	0.4761	0.7951
LOQ	1.4285	1.409

Table 3: Result of Tablet Formulation

Method	Concentra ionin µg/ml		% Label Claim		%RSD		SE	
	MD	**HCTZ**	**MD**	**HCTZ**	**MD**	**HCTZ**	**MD**	**HCTZ**
Method A	25	2.5	99.12±100.8	99.43±100.4	0.71991	0.74194	0.41351	0.42867
Method B	25	2.5	99±100.2	99±99.6	0.61264	0.3077	0.35277	0.1763

n=3

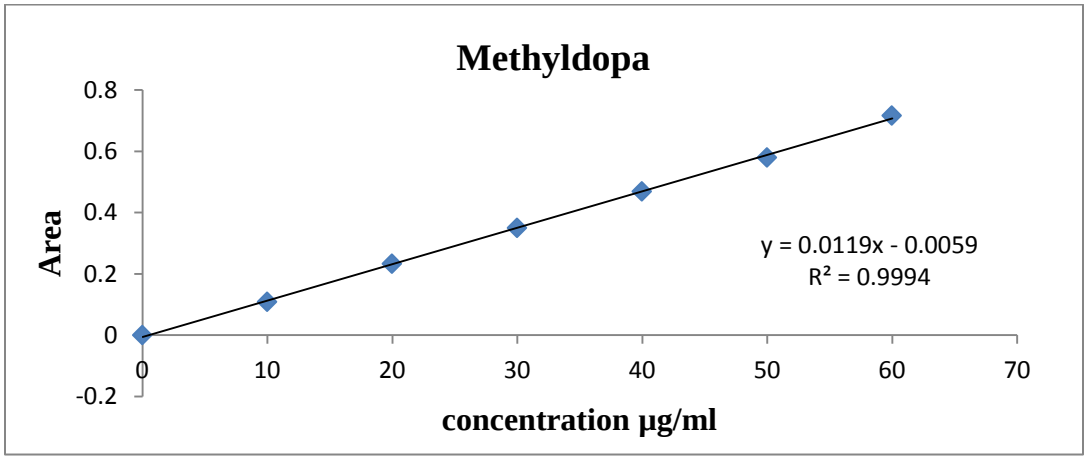

Fig. 3: Calibration Curve of MD AUC Method

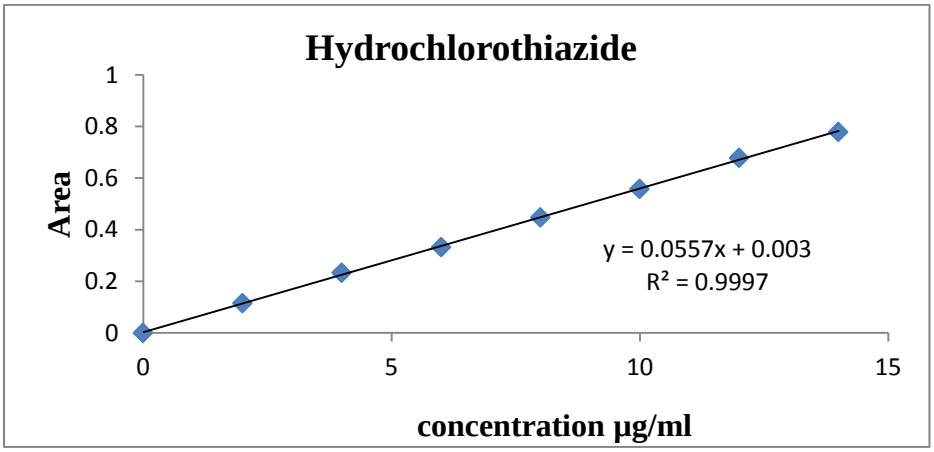

Fig. 4. Calibration Curve of HCTZ

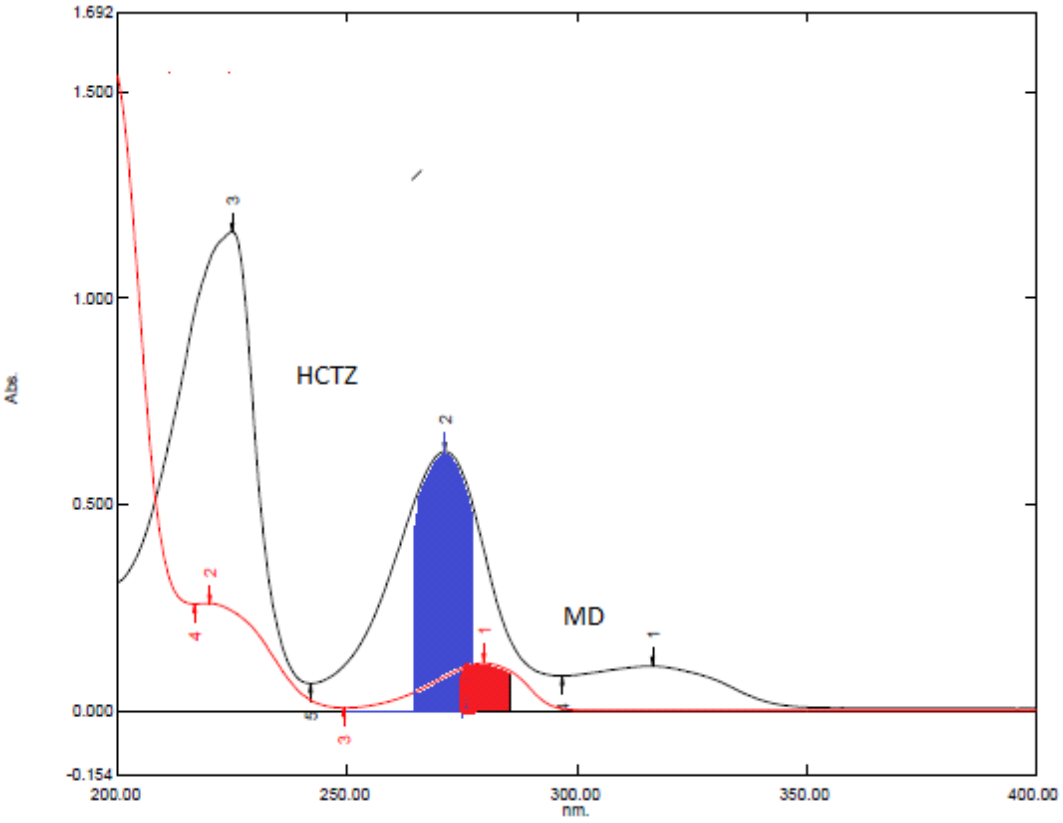

Fig. 5: Overlay Spectra of MD & HCTZ AUC Method

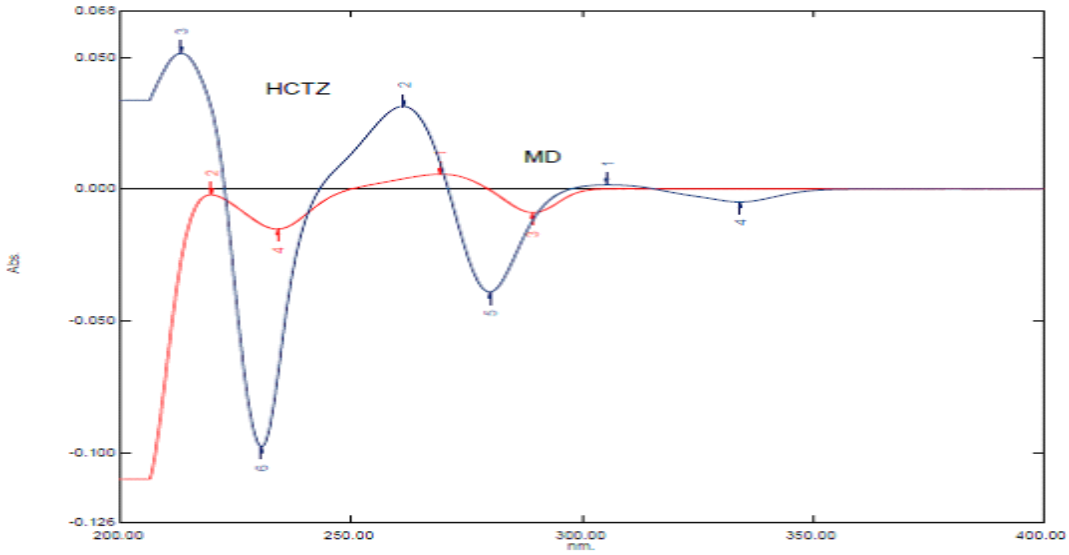

Fig. 6: Overlay Spectra of MD & HCTZ First Order Derivative

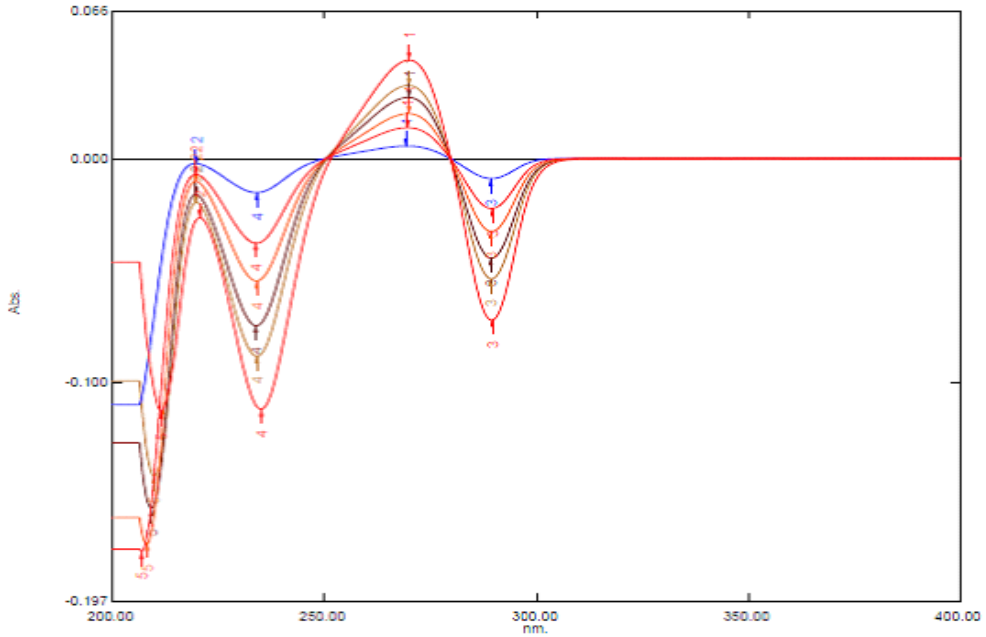

Fig. 7: overlay spectra of MD at 271.40

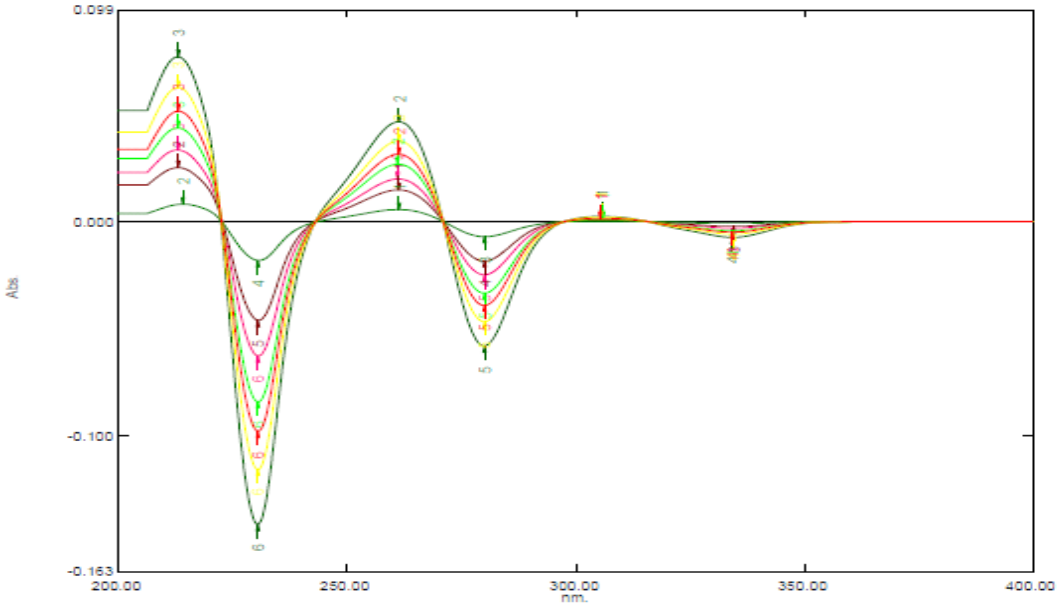

Fig. 8: overlay spectra of HCTZ at 251.20

CONCLUSION

The proposed two spectrophotometric methods were found to be simple, accurate and precise and inexpensive and can be used for routine analysis of Methyldopa and Hydrochlorothiazide in bulk and its formulation.

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