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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF HYDROCHLOROTHIAZIDE, ATENOLOL AND LOSARTAN POTASSIUM IN TABLET DOSAGE FORM

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ABSTRACT
Two UV spectrophotometric methods have been developed for the simultaneous estimation of Hydrochlorothiazide (Hctz), Atenolol (Atn) and Losartan Potassium (Los) in combined tablet dosage forms. The first method involves determination using the Area under curve, the sampling wavelength intervals selected are 274.5-270.5 nm, 226-222 nm and 252-248 nm over the concentration ranges of 0.5-30 mcg mL^{-1}, 1-50 mcg mL^{-1} and 1-60 mcg mL^{-1} for Hctz, Atn and Los respectively. The second method involves determination using the multicomponent mode of the UV visible spectrophotometer, the sampling wavelengths selected are 272.5 , 224 and 250 nm over the concentration ranges of 0.5-30 mcg mL^{-1}, 1-50 mcg mL^{-1} and 1-60 mcg mL^{-1} for Hctz, Atn and Los respectively. The results of the analysis were validated statistically and recovery studies were carried out as per ICH guidelines. The developed methods are simple, rapid, precise, accurate and can be employed for the routine estimation of Hydrochlorothiazide, Atenolol and Losartan Potassium in both bulk and tablet dosage form.

Keywords: *Hydrochlorothiazide; Atenolol; Losartan Potassium; Area under curve method; Multicomponent mode method.*

INTRODUCTION

Hydrochlorothiazide (Hctz) is a diuretic which is official in IP and BP [1-2]. Atenolol (Atn) is a â- blocker which is official in IP, BP and USP [1-3]. Losartan Potassium (Los) belongs to the angiotensin II inhibitor class of drugs and is official in IP [1]. Literature survey reveals several methods such as U.V. spectrosctropy [4-11] and HPLC [12-20] which have been reported for the estimation of individual drugs as well as in combination with other drugs. Not a single UV or HPLC method is reported so far for the simultaneous analysis of Hctz, Atn and Los in their combined dosage form. Hctz, Atn and Los are available in combined tablet dosage form as antihypertensive agents. So a need was felt to develop new methods to analyze the drugs simultaneously. This paper describes two UV spectrophotometric methods for the simultaneous determination of Hctz, Atn and Los in tablet formulation using area under curve method and multicomponent mode method.

EXPERIMENTAL

Material and Methods

A Shimadzu UV/Visible spectrophotometer, model 1700 (Japan) was employed with spectral bandwidth of 2 nm and wavelength accuracy of ± 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. The pure drug samples of Hydrochlorothiazide (Unichem Laboratories, India), Atenolol (Alkem Pharmaceutical, India) and Losartan Potassium (Cipla Ltd, India) were obtained as gift samples. The tablet employed in the study was Losar* Beta-H, (A to z Life Sciences, Pondicherry, India).

Preparation of standard stock solutions

Standard stock solutions (100 mcg mL^{-1}) of Hctz, Atn and Los were prepared by dissolving separately 10 mg of each drug in distilled water.

Preparation of sample stock solutions

Twenty tablets were weighed and crushed to fine powder. An accurately weighed powder sample equivalent to 10 mg of Losartan potassium was transferred to a 100 ml volumetric flask and dissolved in 50 ml of distilled water. After the immediate dissolution, the volume was made up to the mark with the same solvent. The solution was sonicated for about 30 mins and then filtered through Whatmann filter paper No.41. The solution was suitably diluted with distilled water to obtain sample solutions containing Hctz, Atn and Los in the concentrations ratio of 2.5:10:10 mcg mL^{-1} respectively.

Method A: Area under curve method

Standard Stock solutions (100 mcg mL^{-1}) of Hctz, Atn and Los were prepared by dissolving separately 10 mg of each drug in 100 mL distilled water. For forming the simultaneous equations for Area under curve method (AUC), 274.5-270.5 nm, 226-222 nm and 252-248 nm were selected as the three sampling wavelength

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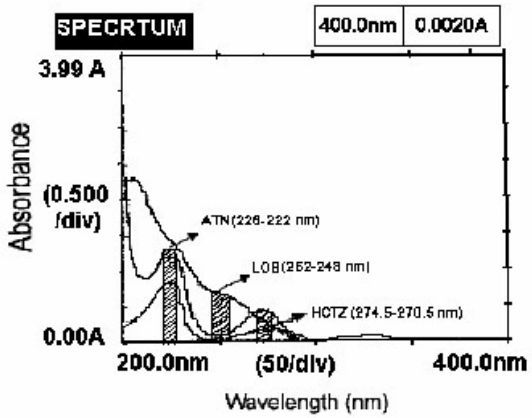
intervals. Figure1 represents the overlain UV spectra of Hctz, Atn and Los. Hctz, Atn and Los exhibited linearity in the concentration range of 0.5-30 mcg mL^{-1}, 1-50 mcg mL^{-1} and 1-60 mcg mL^{-1} at their respective selected wavelength intervals. Co-efficients of correlation were found to be 0.9968, 0.9982 and 0.9996 for Hctz, Atn and Los respectively. The optical characteristics and regression values for the calibration curves are presented in Table 1. For the simultaneous estimation, mixed standards containing Hctz, Atn and Los in the ratio of 1:4:4 were prepared by appropriate dilution of the standard stock solutions. The AUC of the mixed standard solutions were measured at the selected wavelength intervals. A set of three simultaneous equations were established using the mean absorptivity coefficients of Hctz, Atn and Los at the selected wavelength intervals.

Fig.1: *Overlain Spectra of Hctz, Atn and Los.*



A1 = 278 CHCTZ + 21 CATN + 46.6 CLOS …..... (I) at 274.5-270.5 nm ($ë_1$- $ë_2$)

A2 = 541 CHCTZ + 136.9 CATN + 238.5 CLOS…..... (II) at 226-222 nm ($ë_3$- $ë_4$)

A3 = 77.6CHCTZ + 10.8 CATN + 120.9 CLOS.....(III) at 252-248 nm ($ë_5$- $ë_6$)

Where 278, 541 and 77.6 are mean absorbtivity values of HCTZ at ($ë_1$- $ë_2$), ($ë_3$- $ë_4$) and ($ë_5$- $ë_6$) respectively.

21, 136.9 and 10.8 are mean absorbtivity values of ATN at ($ë_1$- $ë_2$), ($ë_3$- $ë_4$) and ($ë_5$- $ë_6$) respectively.

46.6, 238.5 and 120.9 are mean absorbtivity values of LOS at ($ë_1$- $ë_2$), ($ë_3$- $ë_4$) and ($ë_5$- $ë_6$) respectively.

A1, A2 and A3 are the absorbance of mixed standards at ($ë_1$- $ë_2$), ($ë_3$- $ë_4$) and ($ë_5$- $ë_6$) respectively.

CHCTZ, CATN and CLOS are concentrations in g L^{-1}.

The concentration of CHCTZ, CATN and CLOS in mixed standard and tablet formulation can be obtained by solving equation (I), (II) and (III).

Estimation from marketed preparations

Suitable dilutions of tablet sample solutions were scanned in the range of 400–200 nm and its AUC were recorded at the selected wavelength intervals. The concentrations of each drug in sample solutions were calculated using equations (I), (II) and (III).

Method B – Multicomponent mode method

For the analysis of Hctz, Atn and Los by multicomponent method of analysis, the multicomponent mode of the UV visible spectrophotometer was used. Standard stock solution (100 mcg mL^{-1}) of Hctz, Atn and Los were prepared by dissolving separately 10 mg of each drug in distilled water. For multicomponent method of analysis, 272.5 nm, 224 nm and 250 nm were selected as the three sampling wavelengths for Hctz, Atn and Los respectively. The drugs showed linearity in the concentration ranges of 0.5-30 mcg mL^{-1}, 1-50 mcg mL^{-1}, 1-60 mcg mL^{-1} with regression coefficient (r^2) values of 0.9971, 0.9981 and 0.9995 for Hctz, Atn and Los respectively.

Six mixed standards in ratio of 1:4:4 within the Beer's concentration range of Hctz, Atn and Los were prepared by appropriate dilution of standard stock solutions (100 mcg mL^{-1}).

In multicomponent mode of the instrument, the mixed standards were scanned over the range of 190-400 nm at the selected sampling wavelengths. The overlain spectra of the six mixed standards were then employed to determine the concentration of the drugs in sample solutions by analysis of the spectral data of sample solution with reference to that of mixed standards.

Estimation from marketed preparation

Suitable dilutions of tablet sample solution were scanned in the range of 400–200 nm in the multicomponent mode and the concentrations of each component were obtained by the spectral data of sample solutions with reference to that of the pure mixed standards. The analysis procedure was repeated six times with tablet formulation. The developed methods were validated as per ICH guidelines.

The ruggedness of the developed methods was evaluated by performing the analysis employing different instruments and analysts.

RESULTS

The optical characteristics and regression values of the calibration curves for the developed methods are presented in Table 1.

Table 1: *Optical Characteristics and Validation Data of Hydrochlorothiazide, Atenolol and Losartan Potassium*

Parameters	Hydrochlorothiazide		Atenolol		Losartan Potassium	
	Method-A	Method-B	Method-A	Method-B	Method-A	Method-B
Working wavelengths (nm)	274.5-270.5	272.5 nm	226-222	224 nm	252-248	250 nm
Beer-Lamberts Conc. range (mcg mL^{-1})	0.5-30	0.5-30	1-50	1-50	1-60	1-60
Precision*						
Interday (%RSD)						
Intraday (%RSD)	0.9687	0.1230	0.3821	0.171	0.5901	0.459
LOD (mcg mL^{-1})*	0.4939	0.1136	0.3746	0.166	0.6882	0.444
LOQ (mcg mL^{-1})*	0.4619	0.463	0.6158	0.4662	0.3798	0.1987
Regression Values:	0.500	0.500	1.00	1.00	1.00	0.6020
I. Slope*						
II. Intercept*	0.2702	0.0684	0.1294	0.0319	0.1548	0.0382
III. Regression Coefficient (r^2)*	0.1716	0.0411	0.0349	0.0179	0.04833	0.019
	0.9968	0.9971	0.9982	0.9981	0.9996	0.9995
Ruggedness (%RSD)						
Different Instrument	0.442	0.348	0.424	0.329	0.230	0.253
Different Analyst	0.419	0.696	0.435	0.295	0.376	0.247

**Denotes average of six estimations*

Method-A – Area under curve method Method-B – Multicomponent mode method

The mean % content of Hctz, Atn and Los by both methods was 98.86 %, 100.43 % and 100.5 % respectively with low %RSD (less than 2%). Also the mean % recoveries of Hctz, Atn and Los by both methods were 99.58 %, 100.86 % and 100.73 % respectively. The results of the ruggedness study given in Table 1 suggests that the developed methods are unaffected by variations in instruments used and analysts. The results of the proposed methods were also statistically evaluated using t test and F test to determine if there exists any significant difference between the methods. The t-value for Hctz, Atn and Los were found to be 1.228, 0.024 and 1.528 respectively at 10 degrees of freedom and F-value were 3.254, 1.081 and 5.209 respectively at (1, 10) degrees of freedom. The results of the t test and F test also indicated that there is no significant difference between the two methods for the analysis of Hctz, Atn and Los in bulk and tablet formulation.

CONCLUSION

Hydrochlorothiazide, Atenolol and Losartan Potassium are available in combined tablet dosage form for the treatment of Hypertension. Here two simple UV spectrophotometric methods, Area under curve method and Multicomponent method were developed for their simultaneous estimations. The validation studies performed as per ICH guidelines indicated that the proposed methods are suitable for the simultaneous estimation of Hctz, Atn, and Los in pharmaceutical formulations without any interference from the excipients. As the spectrophotometric methods employed distilled water as the solvent, the methods are very economical. The developed methods are simple, rapid, precise, accurate, rugged and can be employed for the routine estimation Hydrochlorotiazide, Atenolol and Losartan Potassium in both bulk and tablet dosage form.

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