eISSN: 2231-0541 CAS CODEN: PHARN8 An EMBASE, EMCare Covered Journal

PHARMANEST

An International Journal of Advances in Pharmaceutical Sciences

Volume 5 | Issue 1 | January-February 2014 | Pages 1850-1858

Original Research Article

ECO-FRIENDLY ANALYTICAL METHOD FOR QUANTITATIVE ESTIMATION OF HYDROCHLOROTHIAZIDE AND TELMISARTAN USING HYDROTROPIC SOLUBILIZING AGENTS

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Received: 17-11-2013 Revised: 23-12-2013

Accepted: 27-12-2013 Available online: 01-01-2014

ABSTRACT

In routine analysis of drugs, lots of organic solvents were used which are costlier, poisonous, and causing atmosphere contamination, so solubility enchantment of poorly water soluble by hydrotropic technique may be a proper choice to preclude the use of organic solvents so that eco-friendly, novel, safe, accurate and precise two methods has been developed for the simultaneous estimation of Hydrochlorothiazide and Telmisartan in tablet dosage form with 2 M Citric acid as hydrotropic solution. It was found that solubility enhanced of HCZ and TEL was more than 46 and 52 fold respectively in hydrotropic solution as compare against with distilled water. HCZ and TEL show maximum absorbances at 271.5 and 291 nm respectively. Citric acid solution did not show any absorbance above 240 nm and thus no interference in the estimation of drugs were seen. HCZ and TEL shows linearity in the concentration range of 5-25 µg/ml and 10-50 µg/ml (r^2= 0.9999 and 0.9991). Method A simultaneous equation method employs 271.5 and 291 nm as two analytical wavelengths, method B is absorption ratio method, which uses 279.8 and 291 nm as two analytical wavelengths were used for estimation of HCZ and TEL. ICH guidelines were used to validated the developed methods. Results of accuracy, precision and other statistical validation analysis were found to be in excellent accordance with the prescribed values and therefore the both methods be able to used for routine monitoring of HCZ and TEL in industry in the assay of bulk drug and as well as tablets dosage form.

Key Words: Hydrochlorothiazide, Telmisartan, Simultaneous equation method, Absorption ratio method, Hydrotropic solubilizing agents.

INTRODUCTION

Hydrochlorothiazide (HCZ) chemically 6-chloro-3,4-dihydro-2H-1,2,4 benzothiadiazine- 7-Sulphonamide 1, 1-dioxide (Fig. 1a), is a thiazide diuretic often considered the prototype member of this class. It has been used in the treatment of edema, hypertension, diabetes insipidus, and hypoparathyroidism[1,2].

Telmisartan (TEL) is chemically (4-((2-n-propyl-4-methyl-6-(1-methyl benzimidazol-2-yl)-benzimidazol-1-yl) methyl)-biphenyl- 2-carboxylic acid), (Fig.1b), a substituted benzimidazole compound, is an AT_1 receptor antagonist which is used to treat hypertension[3,4].

The survey of literature reveals that, HCZ is official in IP[1], USP[2]. Analytical methods that have been reported for the estimation of HCZ in bulk drug and their formulation include UV spectrophotometric and HPLC as single or in combination of Amlodipine Besylate[5,6], Olmesartan

medoxamil[7-12], Nebivolol Hydrochloride[13,14], Eprosartan[15].

TEL is official in IP[1b]. Literature survey reveals that few methods has been reported for determinations of Telmisartan by UV-Vis spectrophotometry[16], HPLC[17-19] and stability indicating HPTLC[20], stability indicating UPLC[21] and forced degradation behavior by RP-HPLC[22].

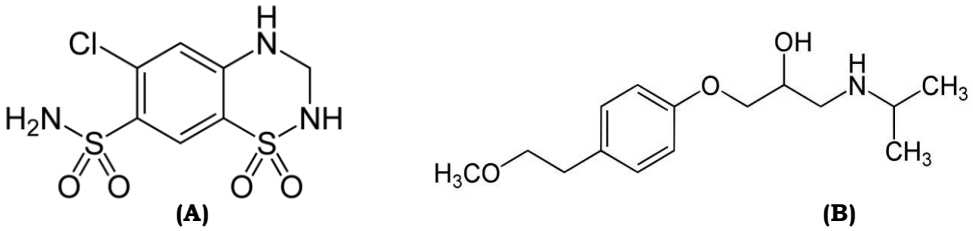

Fig.1. Structure of (A) Hydrochlorothiazide (B) Telmisartan

A hydrotropic technique for solubility is the technique by which aqueous solubility of poorly water soluble drugs and insoluble drugs increases. A variety of techniques have been engaged to enhance the aqueous solubility and hydrotropy is one of them. Sodium salicylate, sodium benzoate, urea, nicotinamide, sodium citrate and sodium acetate are the most common examples of hydrotropic agents utilized to increase the water solubility of drug. Maheshwari[23,24] and Jain[25-40] has analyzed various poorly water-soluble drugs using hydrotropic solubilization phenomenon viz. ketoprofen, frusemide, salicylic acid, pramipexole, torasemide, lomefloxacin, Hydrochlorothiazide, citalopram, amlodipine besylate, ziprasidone, olmesartan medoxamil, levofloxacin,. Various organic solvents such as methanol, dimethyl formamide, chloroform and acetonitrile has been employed for solubilization of poorly water-soluble drugs to carry out spectrophotometric analysis. Drawbacks of organic solvents include their higher cost, toxicity and pollution. Hydrotropic solution may be a proper choice to preclude the use of organic solvents.

Therefore, it was thought worthwhile to employ this mixed hydrotropic solution to extract out the drug from fine powder of tablets to carry out spectrophotometric estimation. There are no reports yet for determination of this combination by proposed methods. Present work emphasizes on the quantitative estimation of HCZ and TEL in their combined dosage form by UV Spectroscopic methods.

MATERIAL AND METHODS

Instrument

UV-Visible double beam double detector spectrophotometer, Shimadzu model-1700 having spectral bandwidth 3 nm and of wavelength accuracy ±1 nm, with 1cm quartz cells was used.

Reagents and chemicals

Reference standard of HCZ and TEL was obtained as gift sample from Matrix Laboratories, Mumbai & Hetero Drugs Ltd., Baddi, and Unichem Laboratories Ltd. Mumbai respectively. Citric acid was obtain from Merck Chemical Division, Mumbai. Reverse Osmosis Water was used during the study.

Preliminary solubility studies of drugs

Solubility of both drugs was determined at 25±1°C. In two screw capped 25 ml of volumetric flask, a definite amount of drug was added with different aqueous systems viz. distilled water and different combination of hydrotropic agent. The volumetric flasks were shaken mechanically for 12 hr. at 25±1°C in a mechanical shaker. These solutions were allowed to equilibrate for next 24 hr. and then centrifuged for 5 min at 2000 rpm. The supernatant liquid was taken for appropriate dilution after filtered through whatman filter paper #41 and analyzed spectrophotometrically against corresponding solvent blank. After analysis, it was found that the enhancement in

the solubility of HCZ and TEL was found to be more than 46 and 52 folds respectively in 2M Citric Acid as compared to solubility studies in other solvents.

Selection of hydrotropic agent

HCZ and TEL was scanned in hydrotropic agent in the spectrum mode over the UV range (200-400) and 2M Citric Acid solution were found to be most appropriate because:

- HCZ and TEL is soluble in it (46 and 52 folds enhancement of solubility)
- HCZ and TEL is stable in hydrotropic agent
- HCZ and TEL, both exhibit good spectral characteristics in it.
- Citric Acid solution has no interference with the λ_{max} of HCZ and TEL, 271.5 nm and 291 nm respectively **Figure 2**

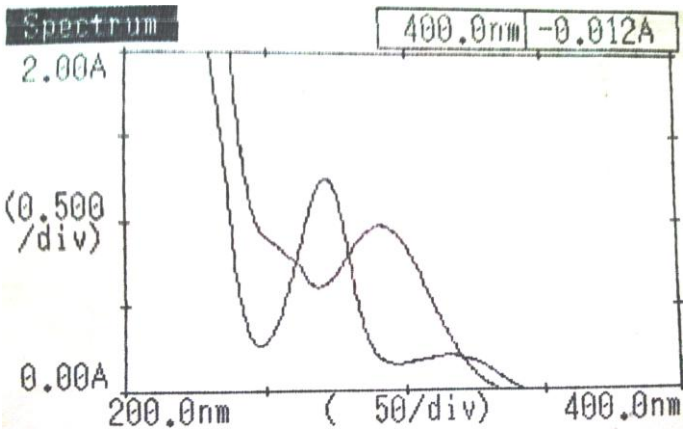

Fig.2.Overlay Spectra of HCZ and TEL in 2 M Citric acid as Hydrotropic

Establishment of stability profile

Stability of HCZ and TEL were established by dissolving in 2M Citric Acid solution used as hydrotropic agent. Solution of HCZ and TEL was scanned under time scan for 30 min. Spectra of drug under time scan shows that drug are stable in hydrotropic solution.

Linearity range and calibration graph

Preparation of Standard Stock Solutions of HCZ and TEL

Standard stock solutions were prepared by dissolving separately 100 mg of each drug in mixed hydrotropic solution and the flask was sonicated for about 10 min to solubilize the drug (Stock-A).

Preparation of Working Standard Solution for calibration curve

The standard solution (1000 µg/ml) was further diluted in ranging from 10-50 µg/ml for HCZ and 10-50 µg/ml for TEL. Calibration curve was plotted between concentrations versus absorbance.

Study of overlay spectra of drugs and selection of method

The spectra exhibit major absorbance maxima at 271.5 nm and 291 nm for HCZ and TEL respectively and isobestic point at 279.8nm. Due to difference in absorbance maxima and having no interference with each other so both drug can be simultaneously estimated by simultaneous equation method (Method A) and Q-analysis method (Method B).

Vierordt's simultaneous equation method (Method A)

The wavelength 271.5 nm (λ_{max} of HCZ) and 291 nm (λ_{max} of TEL) was selected. The absorbencies of HCZ and TEL were measured at 271.5 nm and 291 nm. This method of analysis is based on the absorption of drugs X and Y at the wavelength maxima of the other. The quantification analysis of HCZ and TEL in a binary mixture was performed by using **Eqn-1** and **Eqn-2.** Where C_X and C_Y are the concentrations of HCZ and TEL respectively in the diluted sample, ax_1 and ax_2 are absorptivities of HCZ at λ_1 and λ_2, ay_1 and ay_2 are absorptivities of TEL at λ_1 and λ_2 respectively. A_1 and A_2 are the absorbances of samples at the 271.5 and 291 nm respectively[41].

$C_X = A_2a_{y1} - A_1a_{y2} / a_{x2}a_{y1} - a_{x1}a_{y2}$ **Eqn.1**

$C_Y = A_1a_{x2} - A_2a_{x1} / a_{x2}a_{y1} - a_{x1}a_{y2}$ **Eqn.2**

Q-analysis method (Method B)

In this method absorbances of both the drugs were calculated at two selected wavelengths; among which λ_1 is the wavelength of isoabsorptive point of both drugs and λ_2 is the λ_{max} of either drug among both drugs. From the overlain spectra wavelength 279.8nm (isoabsorption point) and 291 (λ_{max} of TEL) were selected for study. The absorbencies at 279.8nm and 291 nm for HCZ were obtained and similarly for TEL absorbencies are measured at 279.8nm and 291 nm. The concentrations of the individual components were calculated by using the following equations;

$C_X = Q_m - Q_y / Q_x - Q_y) \times A_1 / a_{x1}$ **Eqn.3,**

$C_Y = Q_m - Q_y / Q_y - Q_x) \times A_1 / a_{x1}$ **Eqn.4**

Where, $Q_m = A_2 / A_1$, A_1 is absorbance of sample at isoabsorptive point, A_2 is absorbance of sample at λ_{max} of one of the two components. a_{x1} and a_{x2} represent absorptivities of HCZ at λ_1 and λ_2 and a_{y1} and a_{y2} denote absorptivities of TEL at λ_1 and λ_2 respectively; CX and C_Y be the concentration of HCZ and TEL respectively[41,42].

Table.1.Absorptivities of HCZ (x) and TEL(y) at λ_1 and λ_2

	Method- I				Method- II			
Drug	**274 nm (λ_1)**		**291nm (λ_2)**		**281.2 nm (λ_1)**		**291 nm (λ_2)**	
HCZ	**a_{x1}**	0.0607	**a_{x2}**	0.0136	**a_{x1}**	0.0437	**a_{x2}**	0.0136
TEL	**a_{y1}**	0.0138	**a_{y2}**	0.0377	**a_{y1}**	0.0219	**a_{y2}**	0.0371
					Q_X	0.3112	**Q_Y**	1.6918

N=5

VALIDATION PARAMETERS

The developed methods for simultaneous estimation of HCZ and TEL were validated as per ICH guidelines (Linearity, Accuracy, Precision and Robustness) (ICH, 2005).

Linearity

Linearity of HCZ and TEL was established by response ratios of drug. Response ratio of both drug calculated by dividing the absorbance with respective concentration. Then a graph was plotted between concentration and response ratio**.**

Accuracy

The accuracy of the proposed methods was assessed by recovery studies at three different levels i.e. 80%, 100% and 120%. The recovery studies were carried out by adding known amount of standard solution of HCZ and TEL to pre-analyzed tablet solutions. The resulting solutions were then re-analysed by proposed methods. Total amount of drug found and percentage recovery was calculated.

Precision

Precision of the methods was studied at three levels as at repeatability, intermediate precision (Day to Day and analyst to analyst) and reproducibility.

ANALYSIS OF MARKETED FORMULATION

Twenty marketed tablets of HCZ and TEL Tazloc - H (USV Limited) were weighed and ground to a fine powder; amount equal to 62.5 mg of HCZ was taken in 10 ml volumetric flask. Then 80 ml of hydrotropic agent solution was added and the flask was sonicated for about 10 min to solubilize the drug present in tablet powder and the volume was made up to the mark with hydrotropic solution. After sonication filtration was done through whatman filter paper No. 41. Filtrate was collected and further diluted with hydrotropic agent to get the final concentrations of both drugs in the working range. The absorbances of final dilutions were observed at selected wavelengths and the concentrations were obtained from simultaneous equation method and absorbance ratio method.

RESULTS AND DISCUSSIONS

Based on the solubility and stability and spectral characteristics of the drugs, 2 M Citric acid used as hydrotropic solution. It was found that solubility enhanced of HCZ and TEL was more than 46 and 52 fold respectively in hydrotropic solution as compare with distilled water. HCZ and TEL show maximum absorbances at 271.5 and 291 nm respectively. Citric acid solution did not

show any absorbance above 240 nm and thus no interference in the estimation of drugs were seen. Method-A simultaneous equation method employs **271.5** and **291** nm as two analytical wavelengths, method-B is absorption ratio method, which uses **279.8** and **291** nm as two analytical wavelengths were used for estimation of HCZ and TEL.

Linearity

HCZ and TEL follows the Beer's law in the concentration range of **5-25 µg/ml** and **10-50 µg/ml** (r^2= **0.9999 and 0.9991**). Calibration curve has been shown in Fig 3 and Fig4. Observation of linearity data of both drugs has reported in the Table 2. The Result of their optical characteristics has shown in Table 3.

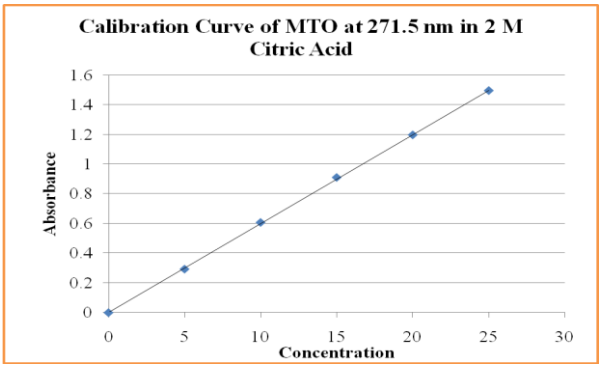

Fig.3.Calibration Curve of HCZ

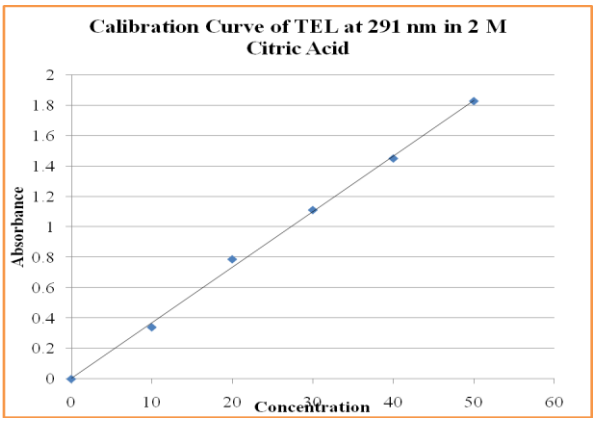

Fig.4.Calibration Curve of TEL

Table.2.Linearity of HCZ and TEL

HCZ (λ_{max} = 271.5 nm)			**TEL(λ_{max} = 291 nm)**		
Standard Conc. (µg/ml)	**Absorbance ± S.D (n=5)**	**% RSD**	**Standard Conc. (µg/ml)**	**Absorbance ± S.D (n=5)**	**% RSD**
0	0	0	**0**	0	0
5	0.293±0.0022	0.75	**10**	0.342±0.004	1.25
10	0.606±0.0028	0.46	**20**	0.788±0.011	1.35
15	0.909±0.0077	0.85	**30**	1.112±0.012	1.09
20	1.195±0.0044	0.36	**40**	1.454±0.015	1.03
25	1.493±0.0201	1.35	**50**	1.828±0.015	0.81

Table.3.Optical Characteristics and Linearity Data of HCZ and TEL

S.No.	Parameters	HCZ	TEL
1	Working λ	271.5 nm	291 nm
2	Beer's law limit (µg/ml)	5-25	10-50
3	Correlation Coefficient (r^2)*	0.9999	0.9991
4	Slope (m)*	0.0599	0.0366
5	Intercept (c)*	0.0011	0.0056

*Average of five determination

Accuracy

The Result of recovery studies at three different levels i.e. 80%, 100% and 120% was calculated. The optimized methods showed good reproducibility and mean recovery with 97.99±0.67 and 98.62±0.57 in method A andn 98.59±1.02 and 98.72±0.55 in method B for HCZ and TEL respectively and result has been reported in Table 4**.**

Table.4.Results of Recovery Studies on Marketed Formulations

Recovery Level %	% Recovery (Mean±SD)*			
	Method A		Method B	
	HCZ	TEL	HCZ	TEL
80	97.22±0.623	98.44±0.412	98.39±1.1	98.49±0.29
100	98.32±0.732	98.83±0.423	98.73±1.2	98.93±0.73
120	98.43±0.640	98.59±0.872	98.64±0.76	98.75±0.64
Mean	**97.99±0.67**	**98.62±0.57**	**98.59±1.02**	**98.72±0.55**

*Average of five determination

Precision

The standard deviation, coefficient of variance and standard error were obtained for HCZ and TEL were satisfactorily low. Result of precision at different level were found be within acceptable limits (RSD<2)Table 5**.**

Table.5.Results of Validation (Mean±SD)

Parameter	Method – A				Method - B			
Precision (Mean±SD)*	HCZ	% RSD	TEL	% RSD	HCZ	% RSD	TEL	% RSD
Repeatability	99.02±0.81	0.82	98.62±0.3	0.30	98.69±0.21	0.21	98.38±0.72	0.73
Day to Day	98.73±0.58	0.59	98.73±0.43	0.44	98.81±0.33	0.33	98.44±0.92	0.93
Analyst to Analyst	98.88±0.37	0.37	99.46±1.1	1.11	98.04±0.57	0.58	99.02±0.92	0.93
Reproducibility	98.65±0.41	0.42	99.98±1.33	1.33	98.92±1.01	1.02	99.21±.0.34	0.34

*Average of five determination

Analysis of marketed formulation

The mean percent label claims of tablet dosage were found to be 98.48±0.839 and 99.30±0.928 in method A, 98.88±1.023 and 97.38±0.676 in method B for HCZ and TEL respectively. The result and statistical evaluation of tablet analysis was reported in Table 6.

Table.6.Results and Statistical Parameters for Tablet Analysis (Tazloc –H)

S.No	Drug	Label Claim	Amount Found	MEAN*	S.D.*	%COV*	Std. Error*
Method A	HCZ	12.5	12.31	98.48	0.839	0.852	0.156
	TEL	40	39.72	99.30	0.928	0.935	0.171
Method B	HCZ	12.5	12.36	98.88	1.023	1.035	0.189
	TEL	40	38.95	97.38	0.676	0.694	0.127

*Average of five determination

CONCLUSION

There was no interference of **2 M Citric Acid** in the estimation and hence the two UV spectrophotometric methods were found to be simple, accurate, economic and rapid for simultaneous estimation of HCZ and TEL in bulk and tablet dosage form. The proposed method can be successfully employed for the routine analysis of HCZ and TEL containing dosage forms.

ACKNOWLEDGMENTS

We are very thankful to M. P. Council of Science & Technology, Bhopal to providing the funding of my research work. I would like to thanks to Sagar Institute of Research & Technology-Pharmacy, Bhopal which provide us the infrastructure and facility of this research work.

CONFLICT OF INTEREST

The Authors declare no conflict of interest

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HOW TO CITE THIS ARTICLE

Surendra Kumar Jain*, Ruchi Jain, Nilesh Jain. (2014 January 1). Eco-Friendly Analytical Method for Quantitative Estimation of Hydrochlorothiazide and Telmisartan Using Hydrotropic Solubilizing Agents.*PHARMANEST,5(1),*1850-1858. http://www.pharmanest.net