



A Stability Indicating RP-HPLC for the Simultaneous Estimation of Hydrochlorothiazide, Amlodipine Besylate and Telemisartan in Bulk and Pharmaceutical Dosage Form

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<http://dx.doi.org/10.13005/ojc/320340>

(Received: March 11, 2016; Accepted: April 17, 2016)

ABSTRACT

A stability indicating RP-HPLC method was developed for the simultaneous estimation of the anti hypertensive drugs Hydrochlorothiazide, Amlodipine Besylate and Telemisartan. These drugs were subjected to stress studies under different conditions as per ICH guidelines. The separations were carried out using C₁₈ reverse phase column (Agilent ODS UG 5 column, 250mm x 4.5mm, 5µm) employing Acetonitrile and Acetate buffer (60:40 v/v) as mobile phase and pH adjusted to 5 at flow rate of 1ml/min was used for separation, detected at 333 nm. The drugs were exposed to acidic, alkaline, oxidative, thermal and photolytic conditions and the stressed samples were analyzed by the proposed method. Degradation studies showed that all the three drugs were degraded under oxidative, thermal and photolytic conditions, negligible degradation observed under acidic, alkaline conditions. Analytical validation parameters such as specificity, linearity, accuracy, precision, Ruggedness and Robustness were determined and relative standard deviation of all the parameters were found to be less than 2%. Hence this method was found to be stable indicator that can be used for the routine analysis of these drugs in the bulk and combined tablet dosage form.

Keywords: Hydrochlorothiazide, Amlodipine Besylate, Telemisartan, RP-HPLC, Stress studies.

INTRODUCTION

Hydrochlorothiazide - C₇H₈ClN₃O₄S₂(fig.1a) (HCT) chemically known as 6-chloro-3, 4-dihydro-2H-1, 2, 4-benzothiadiazine-7-sulphonamide, 1-dioxide. It is a first-line diuretic drug. Amlodipine

besylate -C₂₀H₂₅ClN₂O₅ (fig.1b) (AMB) chemically known as (RS)-3-ethyl 5-methyl 2-[(2-aminoethoxy) methyl]-4-(2-chlorophenyl)-6-methyl-1, 4-dihydropyridine-3, 5-dicarboxylate mono benzene sulfonate. It is used to treat pressure and is a potent dihydro calcium channel blocker. Telemisartan

$C_{33}H_{30}N_4O_2$ (fig. 1c) (TEL) chemically 4'-[(1, 4'-dimethyl-2'-propyl [2, 6'-bi-1H-benzimidazol] - 1'-yl) methyl]-[1, 1'-biphenyl]-2- carboxylic acid. It is also used to treat pressure and an angiotensin converting enzyme inhibitor and angiotensin - II type I receptor blocker^{1,3}. Literature survey reveals that there were many analytical methods for all three drugs alone or in combination with other drugs including spectroscopic and chromatographic methods are reported⁴⁻¹⁰, but there is no method established for the stability indicating RP-HPLC under stress for this combination. The present work describes the development of stability indicating RP-HPLC method, which can quantify these components simultaneously from a combined dosage form. The present RP-HPLC method was validated^{11,12} and applied under stressed conditions according to International conference on harmonization (ICH) guidelines. ICH has made the mandatory need of developing stability indicating assay methods for every drug candidates. There stability indicating assay methods helps in establishing the inherent stability of the drug which provides assurance on detection changes in identity, purity and potency of the product on exposure to various conditions. In the present study Hydrochlorothiazide, Amlodipine Besylate and Telemisartan is exposed to a variety of stress like acidic, basic, thermal, photolytic and oxidative stress conditions¹³. According to ICH guidelines the stress testing of the drug substances helps in identifying the likely degradation products, which in turn can help in establishing the degradation pathways and the intrinsic stability of the molecule. The aim of the present work was to develop stability indicating method for determination of all the three drugs in presence of its degradation products.

EXPERIMENTAL

Instrumentation

Analysis was performed with an Agilent ODS UG 5 column, 250mm x 4.5mm with C_{18} column of injection capacity of 20 μ L. The mobile phase was a 60:40% (v/v) mixture of Acetonitrile: Acetate Buffer (50 mM, pH 5 ± 0.1 , adjusted with orthophosphoric acid). The flow rate was 1.0 mL min^{-1} and the run time was 15 min. Before analysis both the mobile phase and sample solutions were degassed by the use of a sonicator (1.5LH Ultrasonic bath sonicator) and filtered through a 0.45μ membrane filter paper.

The identity of the compounds was established by comparing the retention times of compounds in the sample solution with those in standard solutions. Chromatography was performed in an ambient temperature maintained at $20 \pm 1^\circ\text{C}$. The UV spectrum of HCT, AMB and TEL for selecting the working wavelength of detection was taken using a LAB INDIA double beam UV - Visible spectrophotometer with pair of 10 mm matched quartz cells.

Reagent and chemicals

Pharmaceutically pure sample of HCT, AMB and TEL were obtained as a gift samples from Mylan laboratories. All solvents were of AR grade obtained from S.D. Fine chem. Limited, Mumbai. A combination of HCT (12.5 mg), AMB (5 mg), and TEL (40 mg) in tablet formulation was procured from Indian market TELVAS*-3D, Aristo pharmaceutical Pvt. Ltd, India.

Preparation of Stock Solution of HCT, AMB and TEL

About 50mg of HCT, AMB and TEL were accurately weighed and transferred in to 50 mL volumetric flasks separately. It was dissolved in methanol and the solution was made up to the volume with same solvent.

Method of Validation

The linearity of the prepared method was studied by injecting different concentrations of the HCT, AMB and TEL prepared in the mobile phase in the range of 20-100 μ g/mL and the sample solutions were injected into HPLC system and peak areas were recorded. Precision of the method was demonstrated by interday and intraday variation studies. In the intraday studies analysis of 100% concentration of six replicate injections were performed in a day. In the interday variations studies the analysis of the drug was repeated on three consecutive days. Accuracy of the method was determined by recovery experiments. As known quantity of pure drug was added to the pre analysed sample and the contents were re analysed by the proposed method and percent of recovery was reported.

The limit of detection and limit of quantification were calculated from the slope of calibration curve. Robustness of the method was determined by carrying out the analysis by altering

the conditions of mobile phase composition and flow rate and the effect on the area of peak of intercept and retention times were determined.

Assay of marketed formulation

The contents of twenty commercial tablets were weighed and their mean mass was determined. After grinding the tablets into a fine powder in a glass mortar, an accurately weighed quantity of the tablet powder equivalent to 40 mg of TEL was quantitatively transferred into a 50 ml volumetric flask. The contents were sonicated for 15 min, to ensure the complete solubility of the drug. The solution was made up to the mark with the solvent and filtered through a 0.45 μ membrane filter paper. From the clear solution, further dilutions were made by diluting 1.0 mL into 10 mL with mobile phase; to obtain 100 μ g mL⁻¹ of TEL and 31.25 μ g mL⁻¹ of HCT and 12.5 μ g mL⁻¹ of AMB. Each sample solution was injected and the peak areas were measured for the determination of HCT, AMB and TEL in tablet formulation (table-2).

Degradation Studies

Forced degradation or stress test is undertaken to demonstrate specificity, when developing stability- indicating methods, particularly

when little information is available about potential degradation products. These studies also provide information about the degradation pathways and degradation products that could form during storage. Forced degradation studies may help facilitate pharmaceutical development as well in areas such as formulation development manufacturing and packaging in which knowledge of chemical behaviour can be used improve a drug product¹⁴⁻¹⁶.

Stress Degradation by Hydrolysis under Acidic Conditions

For stability studies 2mL of sample was taken from the HCT, AMB and TEL injection solution into a 100mL volumetric flask and diluted up to the mark using mobile phase

Acid hydrolysis of the drugs were carried out in presence of 1.5N HCl. The drug solution was refluxed with 1.5 N HCl for 2hrs. After exposure for the required duration of time the stressed samples were sonicated and diluted to a concentration of 100 μ g/mL with mobile phase and then diluted to 10 μ g/mL and was injected into the HPLC system after filtration.

Table 1: Results from study of linearity

Parameters	HCT*	AMB*	TEL*
Detection wavelength		333 nm	
Beer's law limit (μ g mL ⁻¹)	20-100	20-100	20-100
Correlation Coefficient (r)	0.999	0.999	0.999
Regression equation (y= mx+c)	Y= 14245x + 40813	Y= 10121x + 15999	Y=11079 x -25536
Slope	14245	10121	11079
Intercept (a)	40813	15999	25536
LOD (μ g mL ⁻¹)	5.2	9.45	7.60
LOQ (μ g mL ⁻¹)	17.16	31.18	25.08

*Mean of six determinations

Table 2: Results from assay of tablet formulation

Amount Present in (mg/tab)			Amount Obtained in (mg/tab)			Label Claim %w/w		
H CZ	AMB	TEL	H CZ	AMB	TEL	H CZ	AMB	TEL
12.5	5	40	12.48	4.99	40.05	99.84	99.8	100.12

Stress Degradation by Hydrolysis under Alkaline Conditions

The alkali hydrolysis of the drug was carried out in 0.1N NaOH and refluxing the solution for 8hrs. After exposure the stressed samples were suitably

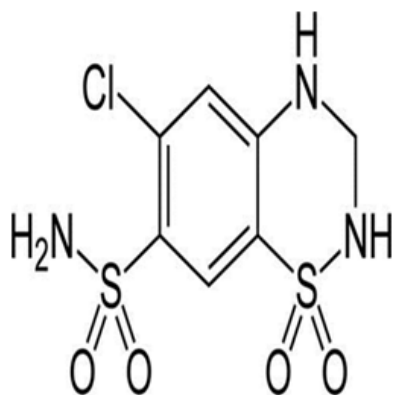


Fig.1(a): Hydrochlorothiazide

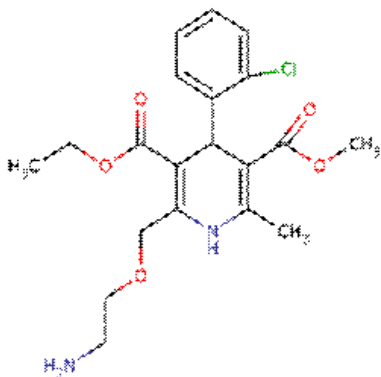


Fig. 1(b): Amlodipine Besylate

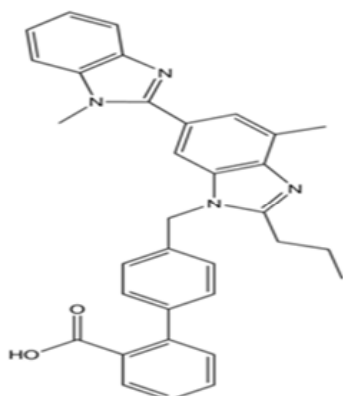


Fig. 1(c): Telmisartan

diluted with mobile phase and then injected into HPLC system.

Oxidative Stress

The drug solution was prepared by dissolving 5mL of HCT, AMB and TEL solution in 10mL of 1% hydrogen peroxide and refluxing the solution at 30°C for 24 hours. After exposure the samples were diluted, filtered and injected into the HPLC system.

Thermal degradation

HCT, AMB and TEL sample solutions was taken in a volumetric flask and kept in an oven maintained at 70°C temperature for 24 hours. Then the sample solution was dissolved and diluted in mobile phase. From this appropriate dilution was made mobile phase and injected in chromatographic system.

Photolytic degradation

5mL of HCT, AMB and TEL samples solution was exposed to ultraviolet lamp in a photostability chamber for 2hrs. After exposure the stressed sample solution was suitably diluted with mobile phase and injected under stabilized chromatographic conditions.

RESULTS AND DISCUSSION

The present study was aimed to develop a simultaneous validated stability indicating RP-HPLC method for the estimation of anti-hypertensive drugs Hydrochlorothiazide, Amlodipine besylate and Telmisartan. After several trails with different mobile phase compositions, a 60:40 (v/v) mixture of Acetonitrile: Acetate Buffer(50 mM, pH 5 ± 0.1, adjusted with orthophosphoric acid) was found to be good. This ratio was optimised as mobile phase for the simultaneous estimation of all the three drugs.

The data obtained from the calibration experiments when subjected to linear regression analysis showed a linear relationship in peak areas and concentration in the range of 20- 100µg/mL for each drug. The developed method was found to be precise as the %RSD values for intra-day and inter-day precision studies were found to be less than 2%. The recovery studies obtained at each added concentration indicates the accuracy of this method.

The limit of detection of HCT, AMB and TEL was found to be 5.2, 9.45, 7.6 $\mu\text{g/mL}$ and limit of quantification was 17.16, 31.18, 25.08 $\mu\text{g/mL}$ respectively thus indicating the sensitivity of the method (table 1). While evaluating the robustness, the peak areas of all the three drugs were found to vary within +0.009%, whereas the retention was found to vary in the range

of 2-8 min. The method was robust as the peak areas and retention time were not significantly affected after varying the parameters like flow rate and wave length. The assay of commercial tablets was established with present chromatographic condition developed and it was found to be more accurate and reliable. The percentage label claim present in tablet formulation

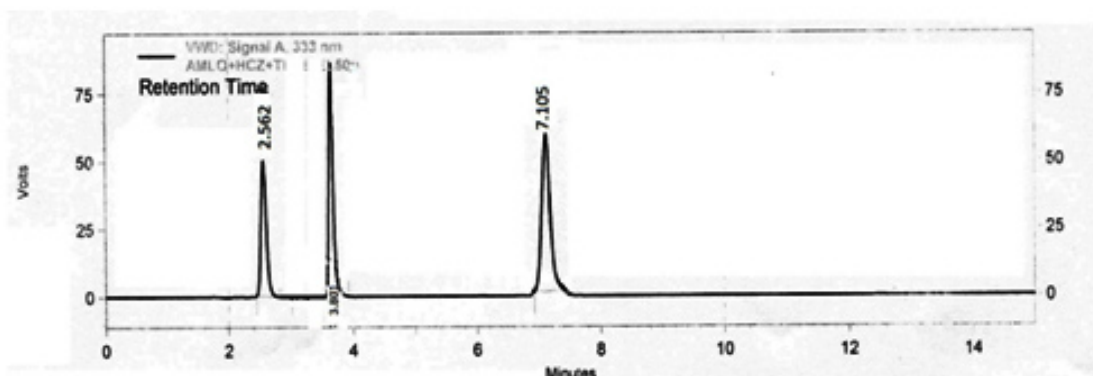


Fig. 2: Optimized chromatogram for HCT, AMB and TEL

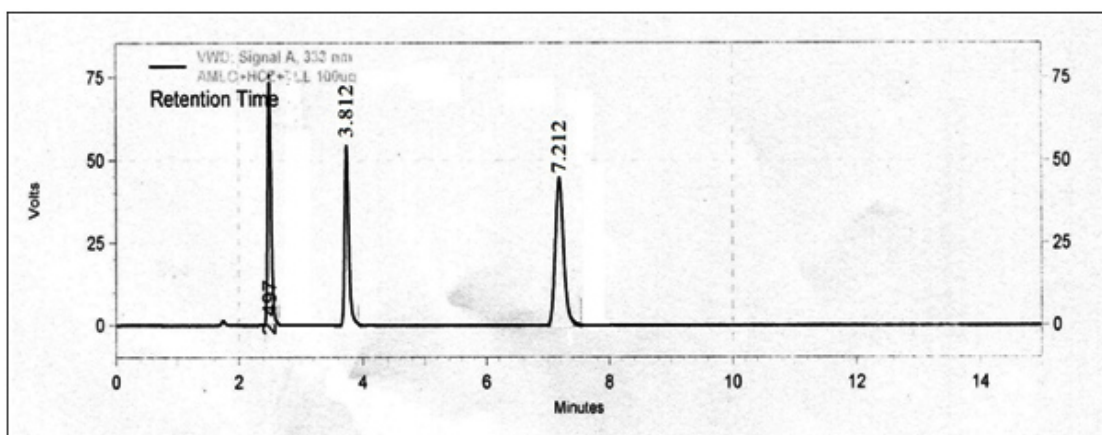


Fig. 3: Chromatogram for Assay of marketed formulation of HCT, AMB and TEL

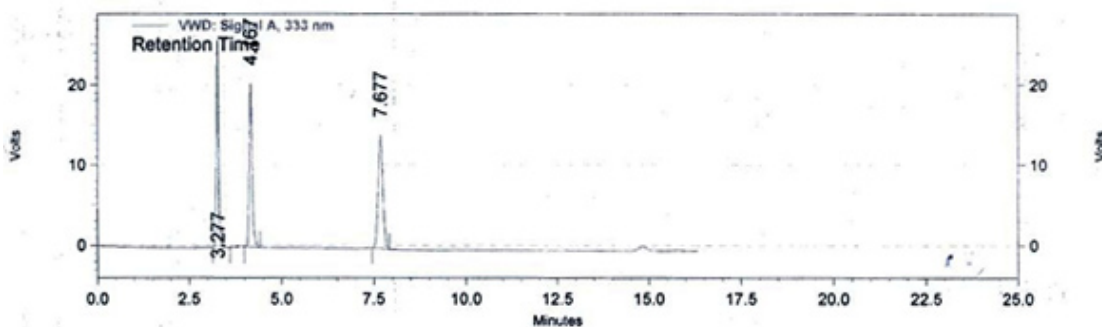


Fig. 4: Chromatogram for Acid Degradation

TELVAS-3D was found to be 99.84, 99.8, 100.12 for HCT, AMB and TEL, respectively (Table 2). The chromatogram for the analysis of formulation is shown in Figure 3.

The drugs were exposed to acidic, base, oxidative, thermal and photolytic conditions and the stressed samples were analysed by the proposed method. Degradation studies showed that all the three drugs were degraded under oxidative,

thermal and photolytic conditions. The drug peak areas decreased sufficiently with drastic change in the R_t values. Two new peaks were observed in chromatograms of acid and alkaline as when compared to the standard, which progressed without any change in R_t . The chromatograms of the stability studies were shown in the figures 4-7.

This present work on the three drugs Viz., HCT, AMB and TEL shows maximum degradation in

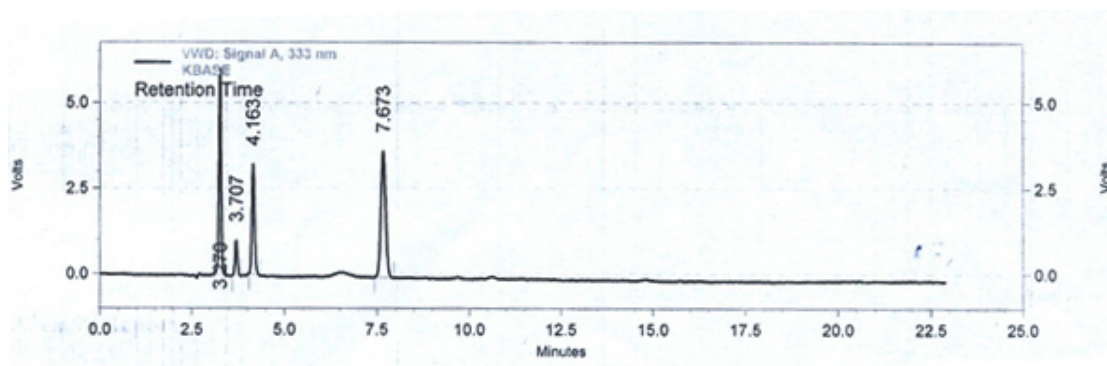


Fig. 5: Chromatogram for Base Degradation

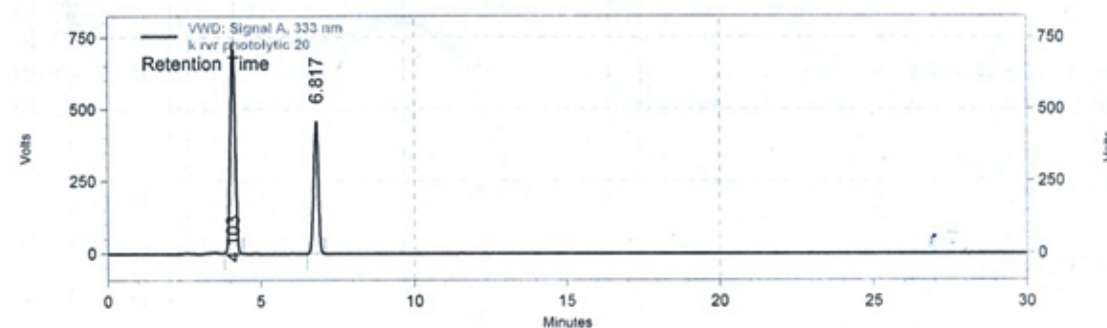


Fig. 6: Chromatogram for Photolytic Degradation

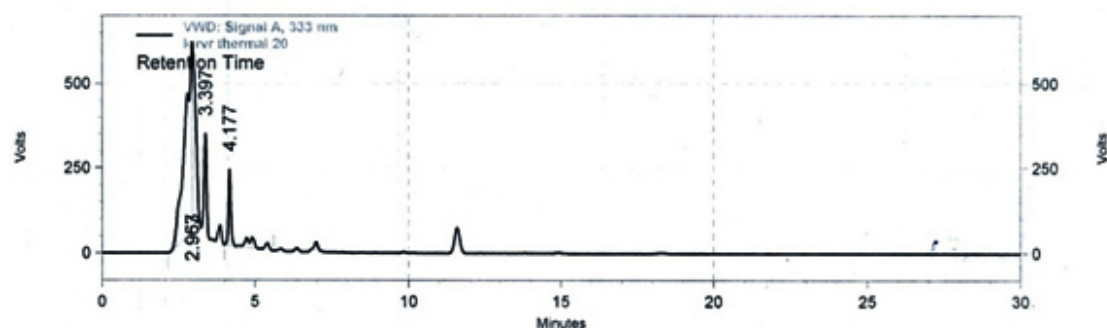


Fig. 7: Chromatogram for Thermal Degradation

photolytic, thermal and oxidative conditions whereas relative stability was noticed when they were exposed to acidic and alkaline conditions. Thus the developed RP-HPLC method was found to be simple, rapid, sensitive, accurate, precise and specific for the simultaneous estimation of all the three drugs in bulk and pharmaceutical dosage forms and for routine analysis in stability studies of these drugs.

CONCLUSION

The HPLC method developed and validated allows a simple and fast quantitative determination of Hydrochlorothiazide, Amlodipine besylate and Telmisartan from its formulation. All the validation parameters were found to be within the limits according to the ICH guidelines. The proposed

method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be suitable for the routine analysis of the marketed formulation.

ACKNOWLEDGEMENTS

The authors are thankful to the Mylan Labs limited, Hyderabad for providing gift sample of Telmisartan, Amlodipine Besylate and Hydrochlorothiazide. The authors also extend their thanks to the management of R.V.R&J.C College of engineering and Chebrolu Hanumaiah Institute of Pharmaceutical Sciences, Chowdavaram, Guntur for providing congenial facilities and support to carry out this research work.

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