TABLE 2: ASSAY OF CVD IN TABLETS

Marketed *Cardivas* tablets	Labelled amount (mg/tablet)	Amount found by proposed method*	Average % recovery by proposed method
Tablet-1	12.5	12.48±.001	99.80
Tablet-2	6.25	6.223±.003	99.56
Tablet-3	3.125	3.118±.012	99.77

*Readings are in the form of mean±sd, where n=3. Tablet-1 is Cardivas of 12.5 mg strength, Tablet-2 is Cardivas of 6.25 mg strength and Tablet-3 is Cardivas of 3.125 mg strength

successfully analyzed by the proposed methods (Table 2). None of the usual excipients employed in the formulation of dosage forms interfered in the analysis of CVD by the proposed methods. In conclusion, the proposed new methods are economic, simple, sensitive, precise and reproducible for the routine determination of CVD in bulk as well as in its pharmaceutical preparations like tablets.

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Simultaneous Reverse Phase Liquid Chromatographic Determination of Metoprolol Tartrate and Hydrochlorthiazide in Tablets

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A simple Reverse phase liquid chromatographic method has been developed and subsequently validated for simultaneous determination of metoprolol tartrate and hydrochlorthiazide in combination. The separation was carried out using a mobile phase consisting of acetate buffer of pH 5.0 and acetonitrile in the ratio 80:20. The column used was Lichrosphere C-18 with flow rate of 1.0 ml/min and UV detection at 254 nm. The described method was linear over a concentration range of 300-700 µg/ml and 40-80 µg/ml for the assay of metoprolol tartrate and hydrochlorothiazide, re-

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spectively. The mean recovery was found to be in the range of 99-102 %. The obtained results confirmed that the method is highly suitable for its intended purpose of separation of metoprolol tartrate and hydrochlorthiazide and its simultaneous determination in formulation.

Metoprolol tartrate and hydrochlorthiazide is a multiingredient formulation, useful in the management of hypertension, heart failure, oedema due to heart failure, and myocardial infarction[1]. As found from literature metoprolol tartrate has been reported to be estimated by densitometric method[2], fluorimetric method[3,4], spectrophotometric method[5-11] and HPLC[12-16]. Hydrochlorthiazide has been reported to be estimated in combination with metoprolol by spectrometry[17] and HPLC[18]. The aim of the present work is to describe a liquid chromatographic procedure for the separation and simultaneous quantification of metoprolol tartrate and hydrochlorthiazide in its formulation.

For the proposed method, acetonitrile-HPLC grade, anhydrous sodium acetate, acetic acid and distilled water (Millipore) were used. The LC system consisted of LC-10 AT pump (Shimadzu), Lichrosphere RP C-18, 5 µm, 250x4.6 mm column, Rheodyne injector equipped with a 20 µl sample loop and UV detector set at 254 nm (Shimadzu SPD-10A VP). The output signal was monitored and integrated using CZ-RA software (Shimadzu). The standard solutions of metoprolol tartrate 1 mg/ml and hydrochlorthiazide 0.125 mg/ml were prepared separately by dilution of the metoprolol tartrate and hydrochlorthiazide respectively in mobile phase (acetate buffer pH 5.0 and acetonitrile in the ratio of 80: 20).

Analysis of marketed sample Betaloc H (Astra Zeneca Pharmaceuticals, Bangalore) of three different batches was carried out. Tablets 20 in number, each containing 100 mg of metoprolol tartrate and 12.5 mg of hydrochlorthiazide were weighed. The tablets were crushed together in a mortar to a fine powder and an amount equivalent to 100 mg of metoprolol tartrate and 12.5 mg of hydrochlorthiazide was transferred into a 100 ml dried volumetric flask. A few drops of acetonitrile were added to dissolve the active solids and then volume made up with mobile phase. The solution was filtered and degassed through membrane filter. The standard and sample solutions were injected separately into stabilized liquid chromatographic system. The retention time for hydrochlorthiazide and metoprolol tartrate at a flow rate of 1 ml/min were recorded as 5.55 and 10.15 min. From the respective areas obtained in standard and sample chromatogram, the amount of contents was calculated. The results of the analysis are tabulated in Table 1.

Accuracy of the method was checked by recovery studies, wherein sample was spiked with known quantity of standard drug of metoprolol tartrate and hydrochlorthiazide at 5 different levels. The percentage recovery ranged from 99.5 to 102% for metoprolol tartrate and 100 to 101.5% for hydrochlorthiazide. The precision of the method was studied by analysis of the mixture and expressed as percentage relative standard deviation which was found to be 1.12% for metoprolol tartrate and 0.84% for hydrochlorthiazide. The linearity of the method was established by analysis of standard solution. The calibration curve was drawn by plotting the peak area versus concentration. The linearity range was found to be 300-700 µg/ml for metoprolol tartrate and 40–80 µg/ml for hydrochlorthiazide. The specificity of the method was established by injecting the placebo. No interference of the placebo was observed with the principal peaks. Hence the method was specific for the combination. Ruggedness of the method was determined by carrying out the experiment on different instruments, by different chemists and on different days. The results showed that the

TABLE 1: RESULTS OF HPLC ASSAY

Marketed sample	Label amount of metoprolol tartrate (mg/tab)	Amount of metoprolol tartrate found* (mg/tab)	Label amount of hydrochlorthiazide (mg/tab)	Amount of hydrochlorthiazide found* (mg/tab)
Bt-1	100	101	12.5	12.6
Bt-2	100	99.9	12.5	12.3
Bt-3	100	101	12.5	12.3

*Average of six determinations. Bt-1, Bt-2 and Bt-3 represent 3 different batches of Betaloc H of Astra Zeneca Pharmaceuticals, Bangalore.

method was rugged as percentage recovery was found to in the range of 99-101%. The robustness of the method was determined by making slight changes in the chromatographic conditions. Buffer pH modification did not have any significant effect. The effect of organic strength on retention time was studied by small change in percentage polarity of mobile phase system and it was found that even slight percentage change in volume or concentration of acetonitrile in the ratio changed the retention time. The system suitability tests were carried out as per USP XXIV requirements. System suitability tests were carried out on freshly prepared standard stock solution of metoprolol tartrate and hydrochlorthiazide and the parameters obtained with 20 µl injection volume. The number of theoretical plates for hydrochlorthiazide and metoprolol tartrate was calculated as 6404 and 9464, respectively. The symmetry factor for hydrochlorthiazide and metoprolol tartrate was 1.25 and 1.17, respectively. The resolution between the two peaks was 2.9. The obtained results confirmed that the method is highly suitable for its intended purpose of separation of metoprolol tartrate and hydrochlorthiazide and its simultaneous determination in formulation.

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Synthesis and Biological Evaluation of 4-(Substituted Aryl)-1-(N-Indolyl Acetamidyl)-3-Chloro-2-Azetidinones

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Various 4-(substituted aryl)-1-(N-indolyl acetamidyl)-3-chloro-2-azetidinones were prepared by condensing N-(arylidine hydrazidomethyl)-indoles with chloroacetyl chloride. Their structures were established by chemical tests, IR and ^{1}HNMR spectral data. These synthesized compounds were tested for their antibacterial, antifungal, antitubercular and antiinflammatory activities.

In continuation of our studies on indoles[1], we report herein the synthesis and biological evaluation of 4-(substituted aryl)-1-(N-indolyl acetamidyl)-3-chloro-2-azetidinones. 2-Azetidinones especially with substituents at 1,4 positions have been found to possess significant antibacterial, antifungal, antitubercular and antiinflammatory activities[2-7]. The incorporation of 4-(substituted aryl)-2-azetidinones moiety to indole framework was thought to enhance the biological activities because the results were quite encouraging when different functionalities were in-

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