maximum absorption at wavelength ($\lambda_{\text{max}}$) 272.8 nm and 260.0 nm is the isoabsorptive point of both drugs. The analytical data for the linearity range shows correlation coefficients of 0.9999 for Olmesartan medoxomil and 0.9991 for HCT at 248.6 nm and 272.8 nm, respectively which is shown in Table 1. For quantitative analysis, concentration of OLM and HCT in tablet sample were determined by using Eqns. 1 and 2, results are shown in Table 2. The detection limits were 0.41 µg/ml for OLM and 0.44 µg/ml for HCT, while quantification limit were 1.25 µg/ml for OLM and 1.33 µg/ml for HCT. The proposed method was validated by performing recovery study. Recovery was in the range of 99.97-100.62 % for OLM and HCT respectively, the standard deviation was found to be less than 2%, shows the high precision of the proposed method. The method can be used for the routine quality control analysis of the OLM and HCT in combined dosage form.

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REFERENCES

RP-HPLC Method for the Estimation of Dutasteride in Tablet Dosage Form

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Patel et al.: RP-HPLC Estimation of Dutasteride in Tablet Dosage Form

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A simple, sensitive and precise RP-HPLC method was developed for the determination of dutasteride in tablet dosage form. The RP-HPLC separation was achieved on phenomenex C18 column (250 mm, id 4.6 mm, 5 µm) using mobile phase methanol:water (90:10 v/v) at a flow rate of 1 ml/min at an ambient temperature. Quantification was achieved with photodiode array detection at 235 nm over the concentration range 1-12 µg/ml. The method was validated statistically and was applied successfully for the determination of dutasteride in tablets.

Key words: Benign prostatic hyperplasia, dutasteride, RP-HPLC

Dutasteride (DTS), selective inhibitor of both, type 1 and type 2 isoforms of 5α-reductase (5-AR) enzyme that converts testosterone to 5α-dihydrotestosterone (DHT) which is responsible for enlargement of prostate, is used in treatment of benign prostatic hyperplasia, frequently occurring in men over the age of 50 years[1]. Chemically, DTS is (5α,17β)-N-{2,5 bis (trifluoromethyl)phenyl}-3-oxo-4-azaandrost-1-ene-17-carboxamide with an empirical formula C27H30F6N2O2, representing a molecular weight of 528.5 g/mol[2].

Literature survey revealed LC-MS and HPLC methods for estimation of DTS in human plasma and pharmaceutical dosage forms[3-5]. A LC-MS-MS method is reported for the simultaneous determination of tamsulosin and dutasteride in human plasma[6]. So it was thought of interest to develop a simple and sensitive RP-HPLC method for determination of DTS in tablet.

All the reagents used were of HPLC grade and analytical grade. Reference standard of DTS was procured from Intaas Pharmaceutical Limited, Ahmedabad with 99.98% purity. Tablets of two different batches of Veltride (0.5 mg) of Intaas Pharmaceutical Ltd. were purchased from a local pharmacy. A standard stock solution of DTS (1 mg/ml) was prepared by dissolving 25 mg of drug in 25 ml methanol. Working standard solution (100 µg/ml) was prepared from stock solution by proper dilution with methanol.

A Shimadzu HPLC (LC-2010HT-liquid chromatograph) equipped with PDA detector (SPD-M20A), phenomenex (Torrance, CA) C18 (250x4.6 mm i.d., 5 µm) column and LC solution software were used. The mobile phase used was methanol:water (90:10, v/v) which was filtered through nylon 0.45 µm membrane filter and degassed by ultrasonication for 15 min.

Linearity of the method was investigated by serially diluting the working standard to give a concentration range of 1-12 µg/ml and 20 µl from this solution was injected. The flow rate was maintained at 1 ml/min.

Temperature of the column was kept at ambient and the effluent was monitored at 235 nm. Calibration curve was constructed by plotting concentration against peak area. The method was validated for linearity, precision, accuracy, and specificity, limit of detection and limit of quantification as per ICH guidelines[7].

Assay of tablets of DTS were performed. Thirty tablets of each batch having 0.5 mg strength were weighed and ground to a fine powder. A quantity of tablet powder equivalent to 10 mg of DTS was transferred to 10 ml volumetric flask, dissolved and diluted with methanol to obtained 1 mg/ml. The solution was sonicated for 15 min and filtered through 0.45 µm membrane filter. The solution was further diluted to obtain concentration 10 µg/ml. Peak area of the above prepared tablet solutions of DTS were measured by using above mentioned chromatographic conditions and the amount of DTS were found from regression equation.

To optimize the HPLC parameters, several mobile phase compositions were tried. Various mobile phases having different ratios of methanol, water and acetonitrile were tried. Drug was retained in mobile phase consisting of acetonitrile:water (60:40, v/v) and methanol:water (60:40, v/v). In acetonitrile:water (90:10, v/v) tailing in the peak was observed. Good peak symmetry and satisfactory retention time was obtained with mobile phase consisting of methanol:water (90:10 v/v). Quantification was achieved with PDA detection at 235 nm based on peak area. The retention time of DTS obtained was 5.2±0.112 (fig. 1). The system suitability tests for HPLC were carried out on freshly prepared solution of DTS (10 µg/ml) and the parameters were studied. The results were summarized in Table 1.

The linear regression data showed a good linear relationship over the concentration range of 1-12 µg/ml as summarised in Table 2. The limit of detection (LOD) and the limit of quantification (LOQ) of
the drug were found by scanning the solution of DTS having different lower concentrations and the LOD and LOQ were found to be 0.5 and 1 µg/ml indicates that the method is sensitive (Table 2). The intraday and interday precision were determined by analyzing standard solution of DTS at three different concentration levels (6, 8, 10 µg/ml). The % RSD for intraday and interday precision was found to be 0.257–0.712% and 0.438-1.080% respectively which indicate that the method is precise (Table 2). Repeatability of the method was studied by injecting 10 µg/ml solution of DTS for six times and peak area was measured and % RSD was calculated which was found to be 0.195 shows repeatability of the method (Table 2). Accuracy of the method was evaluated by standard addition method in which appropriate portions of stock solutions of DTS were spiked into blank placebo matrix to produce concentrations of 80, 100 and 120% of the theoretical concentration. The mean recovery of spiked samples obtained was in range of 98.87-100.31% reveals no interference of excipients and shows that the method is accurate. The proposed validated method was successfully applied to determine DTS in tablet form. The results obtained for tablets of DTS were comparable with the corresponding labelled amounts (0.5 mg/tab) (Table 3). Robustness of the method was estimated by changing the mobile phase composition (3±3), wavelength±1 nm, injection volume (20±2 µl), column temperature (40±3°) and RSD values for all these changes calculated were less than 2 indicate that proposed method is robust. The proposed RP-HPLC method was accurate, precise, sensitive and rapid. The method also can be extended for the routine analysis of DTS in tablet dosage form.

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REFERENCES

Activity of Superoxide Dismutase and Catalase in Fenugreek (Trigonella foenum-graecum) in Response to Carbendazim

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Fenugreek (Trigonella foenum-graecum) is an annual herb, used as a spice and traditionally as medicine. Fenugreek finds its uses in treating hyperglycemia, hyperlipidemia and disorders of gastro-intestinal and cardiovascular systems. Fenugreek cultivation in India is affected by fungal diseases like root-rot and damping-off and fungicides like carbendazim are used to overcome these infections. Fungicides play both positive and negative role in plants; fungicides protect plants from diseases and also exert oxidative stress simultaneously. This report is on the response of antioxidants, superoxide dismutase and catalase in fenugreek seeds and plants treated to different concentrations of carbendazim.

Key words: Carbendazim, catalase, fenugreek, superoxide dismutase

Fenugreek (Trigonella foenum-graecum) is an annual herb and a medicinal plant. The uses of the seeds and leaves of fenugreek are diverse. They are used as spices in food preparations to enhance or impart flavor. Fenugreek seeds are good sources of protein, fat, minerals and dietary fibre[1]. The notable chemical constituents of fenugreek are proteins rich in lysine and tryptophan, flavonoids such as quercetin, trigonelline, saponins, phytic acid and polyphenols [1,2]. The well documented therapeutic uses of fenugreek are its hypoglycemic and hypolipidemic activity[3,4]; fenugreek seed extracts normalize the enhanced lipid peroxidation and relieve oxidative stress by providing antioxidants in diabetic rats[5]. Fenugreek also serves to protect the gastrointestinal [6] and cardiovascular systems[7].

Fenugreek requires a relatively cool climate for its propagation and is highly susceptible to fungal infections. The main diseases that affect fenugreek are foot-rot and damping-off disease in India; leaf spot and powdery mildew are diseases reported to affect fenugreek in other parts of the world[8]. Many different types of fungicides have been evaluated and used to control fungal infections and carbendazim was found to be very effective in controlling foot-rot and damping-off diseases[9]. Fungicides though serve to prevent the incidence of fungal infections and improve quantitative yield, deteriorate the quality of the seeds by altering the composition of seeds and cause undesired biochemical and metabolic changes[10]. This study was aimed at analyzing the effect of carbendazim at different concentrations on germinating fenugreek seeds and plantlets. The response of the antioxidant defenses namely superoxide dismutase (SOD), catalase (CAT) was evaluated and reported in this paper.

Dry fenugreek seeds were purchased from a local grocery. The fungicide used was carbendazim (Bavistin) and was bought from a local chemical

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