

Research Article



DEVELOPMENT OF SPECTROPHOTOMETRIC METHOD FOR SIMULTANEOUS ESTIMATION OF PRODUCTS ACCUMULATED DURING CHOLESTEROL BIOCONVERSION BY *PSEUDOMONAS PUTIDA* MTCC 1259

Kirti Pawar^{1*}, Megha Bhatt¹ and Sambhaji Pawar²

¹Ashok and Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences, Anand, Gujarat, India.

²A R College of Pharmacy, Vallabh Vidya Nagar, Anand, Gujarat, India.

*Corresponding author's E-mail: drkirtipawar@rediffmail.com

Accepted on: 11-06-2011; Finalized on: 25-09-2011.

ABSTRACT

Bioconversion of cholesterol was carried out by growing cells of *Pseudomonas putida* MTCC 1259. Accumulation of two bioconversion products namely cholestenone and 9 α -hydroxy androstadienedione was observed in presence of n-propanol as steroid ring cleavage inhibitor. A spectrophotometric method for simultaneous estimation of the two bioconversion products was developed using sulphuric acid chromogens. The method can be utilized to follow the progress of bioconversion during side chain cleavage of cholesterol by *Pseudomonas putida* MTCC 1259.

Keywords: Cholesterol Side chain cleavage; Simultaneous estimation; Steroid bioconversion; Cholestenone.

INTRODUCTION

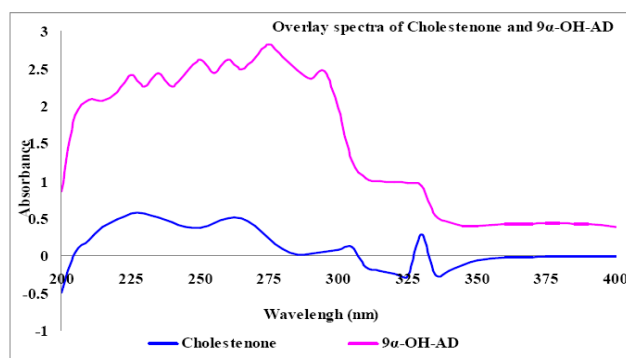
Simultaneous estimation of precursor and product steroids has always been a problem during steroid bioconversions since these compounds show similar absorption patterns under UV light. These compounds form chromogens that show overlapping absorption profiles in visible range. Hence an appropriate estimation method for determination of precursor and product concentrations in the fermentation extracts has been a difficult task. A large number of methods based on HPLC, GLC have been routinely used for the simultaneous estimation of steroids¹. Patil *et al.*, successfully developed a UV method for simultaneous estimation of precursor and product steroids during bioconversion of Androstenedione². Pawar *et al.*, developed a spectrophotometric simultaneous estimation method for hydrocortisone succinate and its bioconversion product³. Sulphuric acid chromogens have been effectively used for simultaneous determination of steroidal compounds on thin layer chromatography. *Pseudomonas putida* MTCC 1259 was found to accumulate two bioconversion products identified to be cholestenone and 9 α -hydroxy androstadienedione (9 α -OH-AD) during cholesterol bioconversion in presence of n-propanol as inhibitor for steroid ring cleavage⁴. Further, the two products were the only steroids detected in the fermentation medium after about 72 hrs of bioconversion in presence of n-propanol. It was decided to prepare sulphuric acid chromogens of the side chain degradation products of cholesterol and study their absorption spectra with a view to develop a simultaneous estimation method for the two bioconversion products.

MATERIALS AND METHODS

Pseudomonas putida MTCC 1259 was grown in a medium containing (g l⁻¹): Peptone, 5; Yeast extract, 2; Beef extract, 1 and Sodium Chloride, 5; pH of the medium was

adjusted to 7.0; dispensed (20 ml) in a series of 100 ml conical flasks and sterilized by autoclaving at 121°C for 15 min and allowed to cool to room temperature. The flasks were inoculated with actively growing culture of the organism in the same medium, allowed to grow overnight at 30°C on a shaking platform at 100 rpm and cholesterol (100 μ g) dissolved in 0.2 ml acetone was added. After 24 hr of addition of substrate cholesterol, n-propanol was added to the bioconversion flasks to achieve a 5 % concentration. Samples were drawn at specified intervals and extracted twice with equal volumes of ethyl acetate. The organic layer was decanted, dried over sodium sulphate and solvent evaporated by keeping the tubes in a boiling water bath. The bioconversion products were determined qualitatively by Thin Layer Chromatography.

For isolation of the steroidal fermentation products, the contents of the flasks were extracted with twice the volume of ethyl acetate, separated by preparative TLC⁵, and re-crystallized from acetone or methanol and used for further studies. The purified bioconversion products (30 μ g) were separately dissolved in 3 ml concentrated sulphuric acid, chromogens developed by heating the tubes in boiling water bath for 30 minutes and absorption spectrum of the compounds determined in the wavelength range of 200 to 400 nm.



RESULTS AND DISCUSSION

It is clear from the above figure that both the chromogens show significant absorbance at all the wavelengths selected for drawing the spectrum. It was decided to make equimolar solutions of the compounds and determine the absorptions of the respective sulphuric acid chromogens to determine their difference in absorptivities. Using the above graph, absorption maxima of 9 α -OH-AD was at 280 nm & that of cholestenone was chosen as 260 nm. Simultaneous estimation equations for cholestenone and 9 α -OH-AD were developed using the method described for simultaneous estimation of solasodine and solasonine⁶. Following simultaneous equations were used for developing equations for estimation of 9 α -OH-AD and cholestenone in binary mixture.

$$X_1 = C_d \alpha_1 + C_n \beta_2 \dots\dots\dots (1)$$

$$X_2 = C_d \alpha_2 + C_n \beta_1 \dots\dots\dots (2)$$

Where, X_1 = absorbance of binary mixture at 280 nm; X_2 = absorbance of binary mixture at 260 nm; C_d = % concentration of cholestenone (cholestenone λ_{max} = 260 nm); C_n = % concentration of AD (AD λ_{max} = 280nm); α_1 = Mass Absorptivity of AD at 280 nm = 886; β_2 = Mass Absorptivity of cholestenone at 280 nm = 27.3; α_2 = Mass Absorptivity of AD at 260 nm = 873.33; β_1 = Mass Absorptivity of cholestenone at 260 nm = 167

Absorptivity of the 30 μ g/ml solutions of the two compounds was determined, values put in the above equations and following equations were developed for determination of concentration of the two compounds in the binary mixtures.

$$C_n = 0.00713824 \times A_{260} - 0.00703544 \times A_{280} \dots\dots\dots (A)$$

$$C_d = 0.0013457 \times A_{280} - 2.1994416 \times A_{260} \dots\dots\dots (B)$$

Where, A_{280} = Absorption of binary mixture at 280 nm and A_{260} = Absorption of binary mixture at 260 nm. A simultaneous estimation method for the two bioconversion products of cholesterol by was successfully developed.

Acknowledgements: The authors thank Charutar Vidyamandal, Vallabh Vidya Nagar, Anand, Gujarat, India

for extending all the facilities for successful completion of this work. The authors like to acknowledge Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India for providing the strains used for the study and Sophisticated Instrumentation Centre for Applied Research and Training (SICART), Vallabh Vidya Nagar, Anand, Gujarat, India for the IR and Mass Spectral analysis of steroid samples.

REFERENCES

- Owen Robert W., Arthur N. Mason and Rodney F. Bilton. The degradation of β -sitosterol by pseudomonas sp. NCIB 10590 under aerobic conditions. Journal of Lipid Research, 34: 1983, 1500-1511.
- Patil S., Srivastava A., Shukla A, and Phase N. Spectrometric estimation of 4-androstene, 3,17-dione and 1,4-androsta3,17-dione during C1,2-dehydrogenation by *Mycobacterium fortuitum* NRRL B 8153; World Journal of Microbiology and Biotechnology, 7, 1991, 626-627.
- Pawar Kirti, Lad Neelam and Pawar S. P.: Simultaneous spectrophotometric estimation of 11 β ,17 α -dihydroxy -4-pregnene-3,20-dione- 21-succinate and its 1(2)-dehydrogenated product during bioconversion by *Comamonas acidovorans* MTCC 3364. Recent Research in Science and Technology 2(11): 2010, 01-04.
- Bhatt Megha Side Chain Cleavage of Cholesterol by some microorganisms. M. Sc. Dissertation Thesis, Sardar Patel University, Vallabh Vidya Nagar, Anand, Gujarat, India. 2011.
- Shinde K: Biotransformation of some steroid drug precursors to 17-ketosteroids by mixed bacterial culture. Ph. D Thesis, Devi Ahilya Vishwavidyalaya, Indore (MP) India. 2000.
- Patil S., Srivastava A., Shukla A, and Phase N. Spectrophotometric determination of solasonine and its bioconversion product solasodine; Indian Journal of Chemistry, 32B, 1992, 1083-1085.

