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SIMULTANEOUS SPECTROPHOTOMETRIC ESTIMATION OF 11β,17α-DIHYDROXY -4-PREGNENE-3,20-DIONE- 21-SUCCINATE AND ITS 1(2)-DEHYDROGENATED PRODUCT DURING BIOCONVERSION BY *COMAMONAS ACIDOVORANS* MTCC 3364

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Abstract

A spectrophotometric method for simultaneously estimating 11β,17α-dihydroxy-4-pregnene-3,20-dione 21-succinate and its 1(2)-dehydrogenated product has been developed using sulphuric acid chromogens. The method was used to follow the progress of bioconversion during C-1(2)-dehydrogenation by *Comamonas acidovorans* MTCC 3364.

Keywords: Steroid 1(2)-dehydrogenation; Simultaneous estimation; Steroid bioconversion; Hydrocortisone; Prednisolone estimation

Introduction

Two corticosteroids, 11β,17α-dihydroxy-4-pregnene-3,20-dione-21-succinate (hydrocortisone succinate) and its dehydrogenated product 11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione-21-succinate (prednisolone succinate) are important anti-inflammatory agents used in variety of dosage forms. Simultaneous estimation of precursor and products during steroid 1(2)-dehydrogenation poses a difficult task. Most of the described methods utilize sophisticated, sensitive but expensive techniques like High Performance Liquid Chromatography and Gas Liquid Chromatography (Kaul & Mattiasson, 1986, Pinhero and Cabral, 1990).

The present work reports a rapid and cost effective spectrophotometric method for routine estimation of precursor and product during C-1(2)-dehydrogenation of hydrocortisone succinate by *Comamonas acidovorans* MTCC 3364. The method is based on characteristic chromogen formation by the steroids which show concentration dependent difference in absorption at the selected wavelength.

Materials and Methods

Ethyl acetate solutions of hydrocortisone succinate and prednisolone succinate containing 25 µg of compound were vacuum dried in separate tubes. The residue was dissolved in 3.0 ml 40%, 60% and 98% sulphuric acid, tubes kept in boiling water bath for different time intervals and cooled to room temperature. Absorption spectra of the developed chromogen were recorded between 250 and 650 nm. To select the best wavelength for simultaneous determination of the compounds, a series of tubes containing 10 to 100 µg compounds were taken, chromogens prepared and absorbance determined at selected wavelengths.

Comamonas acidovorans MTCC 3364 was grown in a medium containing (g l^{-1}): $(NH_4)_2SO_4$, 3; $CaCl_2$, 0.2; $FeSO_4$, 0.1; Glucose, 20; K_2HPO_4, 3.0; KH_2PO_4, 1.0; $MgSO_4$, 0.2; pH adjusted to 7.0 was used for bioconversion of hydrocortisone succinate. Medium (20 ml) was dispensed in a series of 100 ml conical flasks and sterilized at 121 ºC for 15 min and allowed to cool to room temperature. The flasks were inoculated with actively growing culture of the strains of microorganism in the same medium, allowed to grow overnight at 30 ºC on a shaking platform at 100 rpm and hydrocortisone succinate dissolved in 0.2 ml acetone was added. 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 residues were dissolved in 3 ml sulphuric acid, chromogen developed, absorbance determined at selected wavelength and mole % conversion was calculated.

Results and Discussion

The sulphuric acid chromogens showed stable absorbance in 98% sulphuric acid after 30 min reaction in boiling water bath. The overlay spectra (fig 1) of the two compounds showed minimum interference of

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hydrocortisone succinate after 400 nm. To determine the best wavelength for estimation, absorbance of chromogens was determined at 400, 460 and 545 nm (fig 2 & 3) and difference in absorbance at each concentration determined. Maximum difference was observed at 545 nm at highest concentration of the chosen range and hence selected for determination of the appearance of product in the bioconversion medium.

Fig 1: Overlay spectra of Hydrocortisone succinate and Prednisolone succinate

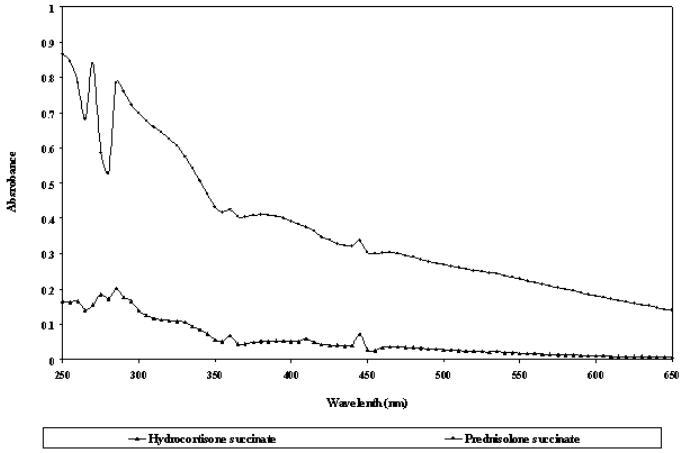


Fig 2: Absorbance of different amounts of hydrocortisone succinate at selected wavelengths

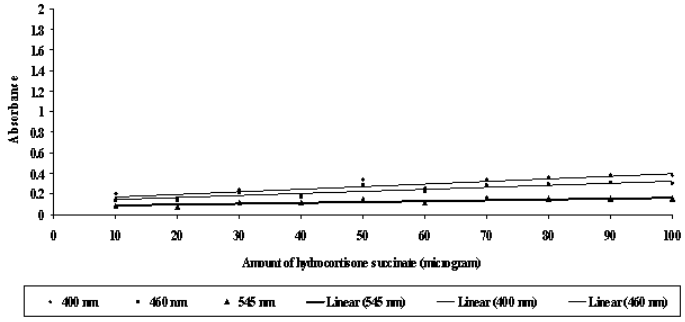
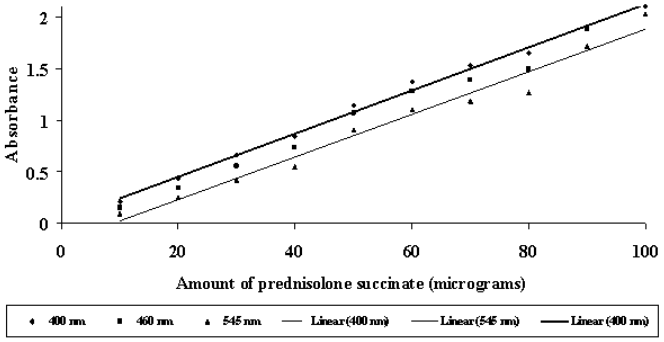


Fig 3: Absorbance of different amounts of prednisolone succinate at selected wavelengths



The concentration of the precursor was not allowed to increase above 100 µg ml $^{-1}$ in the extracts of bioconversion media and compensated by appropriate dilution of extract before producing sulphuric acid chromogens whenever higher substrate concentrations had to be added. Similar simultaneous estimation method for Androstenedione and its dehydrogenated product has been developed by Patil et al (1991) and for solasonine and solasodine estimation by Patil et al (1992) and successfully applied for simultaneous estimation of precursor and product steroids during bioconversion.

The mole % conversion hydrocortisone succinate bioconversion by *Comamonas acidovorans* MTCC 3364 using different substrate concentrations have been shown in table 1. The maximum accumulation of product was observed at 0.5 mg l $^{-1}$ substrate concentration after 72 hr incubation. Thin layer chromatography revealed rapid disappearance of the accumulated product after achieving maximum bioconversion. The production of prednisolone succinate decreased with increasing substrate concentration. Nadia Naima et al. (2003) used *Pseudomonas fluorescens* cells entrapped in poly-hydroxyethylmethacrylate (Poly-HEMA), which converted hydrocortisone to prednisolone with maximum bioconversion efficiency at a substrate concentration of 2 mg l^{-1} medium. The result also complies with observation of Jiradej Manosroi et al. (1998) who reported reduction in prednisolone production with increasing hydrocortisone concentration during bioconversion by immobilized cells of a three bacterial strains (*Bacillus sphaericus* ATCC 13805, *Bacillus sphaericus* SRP III and *Arthrobacter simplex* 6946) in an aqueous medium. There was decrease in amount of accumulated product on further incubation with the organism indicating degradation to non steroidal compounds. Similar reduction of accumulated steroidal products has been reported by Patil et al (1991).

Table 1: Mole % conversion of hydrocortisone succinate by growing cells of *Comamonas acidovorans* MTCC 3364

Substrate concentration (mg l^{-1})	Mole % conversion after incubation (hr)		
	24 hrs	48 hrs	72 hrs
0.5	31.68 ± 3.0	47.85 ± 2.3	69.55 ± 1.8
1.0	63.00 ± 1.7	41.65 ± 1.4	56.80 ± 1.1
1.5	50.88 ± 5.5	17.39 ± 5.7	35.46 ± 5.7
2.0	09.81 ± 1.5	37.37 ± 0.9	37.40 ± 1.1
5.0	09.81 ± 1.1	10.99 ± 0.5	05.75 ± 2.7

Data are mean of three replicates ± SD

Comamonas acidovorans MTCC 3364 was found to 1,2-dehydrogenate hydrocortisone succinate effectively as an actively growing culture The results do not coincide with the findings of Srivastava and Patil (1998) who indicated the dependence of steroid 1(2) dehydrogenation on the C-17 side chain during cholesterol metabolism by immobilized *Mycobactrium fortuitum*. It can be indicated that 1(2) dehydrogenation of hydrocortisone succinate by *Comamonas acidovorans* MTCC 3364 is not affected by presence of aliphatic side chain at C-17 position.

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