

Novel approach for downstream processing of phytosterol bioconversion

Sergey M Khomutov,^{a,*} Andrey A Shutov,^a Aleksey D Averin,^b Dmitry V Dovbnya^a and Marina V Donova^a



Abstract

BACKGROUND: Downstream processing is an important part of industrial steroid biotechnology. The traditional recovery methods require significant amounts of the organic solvents which are associated with environmental risks. There is a need for environmentally friendly downstream processing which also may open the prospects for regeneration and repeated use of the costly materials.

RESULTS: Microbial conversion of phytosterol to C17-ketosteroids in methylated cyclodextrin (mCD) solutions was studied as a model bioprocess. Quantitative aspects of competitive complexation of mCD and natural β -CD (β -CD) with steroids were studied under different temperature modes and CD concentrations. The method of steroid recovery based on precipitation of insoluble β -CD complexes ensured the complete isolation of androst-4-ene-3,17-dione, androsta-1,4-diene-3,17-dione, 9 α -hydroxyandrost-4-ene-3,17-dione, 20-hydroxymethyl-pregna-1,4-dien-3-one and 3 β -hydroxyandrost-5-en-17-one from aqueous media. Analysis of the ¹H nuclear magnetic resonance (NMR) spectra showed a constant molar ratio of β -CD to steroid equal to 2 in the obtained steroid β -CD complexes. The extraction of the steroid from the β -CD complex by small volumes of organic solvent provided a quantitative yield. The method developed ensures regeneration and repeated use of the mCDs without decrease of the basic bioconversion outputs, as was demonstrated in a series of phytosterol to androsta-1,4-diene-3,17-dione bioconversions by *Mycolicibacterium neoaurum* VKM Ac-1816D.

CONCLUSIONS: Downstream processing based on manipulation of steroid solubility in aqueous cyclodextrin solutions is a promising alternative to traditional steroid extraction with organic solvents. This environmentally friendly approach ensures re-use of materials without decrease in bioconversion outputs.

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Supporting information may be found in the online version of this article.

Keywords: downstream; bioconversion; recycling; bacteria; extraction

ABBREVIATIONS

7 α ,15 α -dihydro-DHEA	3 β ,7 α ,15 α -trihydroxy-androst-5-en-17-one
7 α -OH-DHEA	3 β ,7 α -dihydroxy-androst-5-en-17-one
9-OH-AD	9 α -hydroxyandrost-4-ene-3,17-dione
AD	androst-4-ene-3,17-dione
ADD	androsta-1,4-diene-3,17-dione
CD	cyclodextrin
DHEA	dehydroepiandrosterone, 3 β -hydroxyandrost-5-en-17-one
HMPD	20-hydroxymethyl-pregna-1,4-dien-3-one
mCD	randomly methylated β -cyclodextrin
β -CD	natural β -cyclodextrin

INTRODUCTION

A growing need for efficient microbial transforming systems currently exists in the pharmaceutical industry for the commercial production of steroids. The production of many valuable steroids is based mainly on bioconversion of plant sterols—phytosterols.¹ A significant number of modern technologies for the combined

chemical–microbial synthesis of steroid hormones are known, which include specific bioconversion of phytosterol and key steroid intermediates.² The microbial transformation of phytosterol and other steroids is complicated by their hydrophobicity, which limits their accessibility to microorganisms. Extraordinary hydrophobic properties and low rates of steroid transfer into and out of the cell are among the major limiting factors in steroid bioconversion.

Most methods of ‘reconciliation’ of hydrophobic substrates with hydrophilic biocatalysts operating in an aqueous medium involve

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Downstream A Novel

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