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Short Communication

Determination of Lovastatin (mevinolin) and mevinolinic acid in fermentation liquids

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ABSTRACT

A rapid and simple HPLC method for the determination of Lovastatin (mevinolin) and mevinolinic acid in fermentation fluids of ***Aspergillus terreus*** using a Separon SGX C_{18} column and methanol-18 m*M* orthophosphoric acid (77.5:22.5, v/v) as mobile phase with detection at 238 nm is described. The detection limit of Lovastatin and mevinolinic acid was 20-30 ng/ml.

INTRODUCTION

Lovastatin (1′,2′,6′,7′,8′,8a′-hexahydro-3,5-dihydroxy-2′,6′-dimethyl-8′-(2″-methyl-1″-oxobutyl)-l-naphthaleneheptanoic acid 5-lactone) (Fig. 1) is a very potent hypocholesterolaemic drug (mevinolin, Mevacor). It is produced by fungi of the genera *Monascus*, *Aspergillus* and *Penicillium* [1]. The biologically active substance is mevinolinic acid, into which Lovastatin is converted *in vivo* [2].

Lovastatin and mevinolinic acid have been separated as trimethylsilyl derivatives by gas chromatography and identified by mass spectrometry [3]. Reversed-phase HPLC has been used for ethyl acetate extracts of fermentation liquids, blood plasma and bile analysis. The substances were separated as their 4-nitrobenzoyl derivatives isocraticaly [4] or without derivatization by gradient elution (for bile samples) [5].

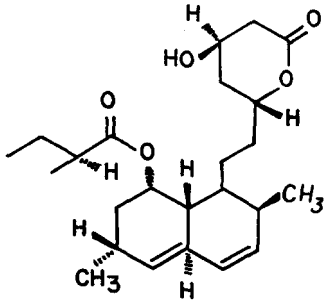

Fig. 1. Structure of Lovastatin.

In this paper, a rapid method for the determination of Lovastatin and mevinolinic acid under iso-

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cratic conditions and without preliminary sample treatment and derivatization is described.

EXPERIMENTAL

Chemicals

Lovastatin (mevinolin) was obtained from Sigma (St. Louis, MO, USA). Mevinolinic acid was prepared by alkaline hydrolysis of Lovastatin according to Endo *et al.* [6]. Methanol was of HPLC purity (Pierce, Rockford, IL, USA). All other chemicals were of at least analytical-reagent grade from Lachema (Brno, Czechoslovakia).

Sample treatment

The samples of fermentation broth were centrifuged at 4500 g for 5 min and the clear supernatant (10 μl) was injected directly into the HPLC column.

Cultivation

Aspergillus terreus ATCC 20542 [1] for Lovastatin production was cultivated at 28°C on a rotary shaker in conical flasks with 40 ml of medium. Inoculum (24 h) was prepared on the following medium: corn steep 5, tomato paste 40, oat meal 10, glucose 10 g/l, and trace elements [1], (pH 6.8), and 5-10% of the inoculum was transferred into the production medium: glucose 45, milk peptone 24, yeast extract 2.5 and polyethylene glycol P2000 2.5 g/l, (pH 7.4) and cultivated for 5 days (maximum production) [1]. This cultivation liquid was used directly for Lovastatin and mevinolinic acid analyses.

Instruments

The HPLC system was composed of and LC-3B high-pressure pump (Perkin-Elmer, Norwalk, CT, USA) combined with a Rheodyne Model 7125 injection valve with a 10-μl sample loop and a Model 990 + diode-array detector (Waters, Milford, MA, USA), set at 238 nm. A glass column (150 × 3 mm I.D.) packed with Separon SGX C_{18}, 5 μm (Tessek, Prague, Czechoslovakia) was used. The compounds were eluted isocraticaly with methanol-18 m*M* orthophosphoric acid (77.5:22.5, v/v) at a flow-rate of 0.5 ml/min. Spectral scanning from 200 to 350 nm was used during method development.

RESULTS AND DISCUSSION

The aim of this work was to develop a rapid method for the determination of Lovastatin and mevinolinic acid in fermentation broths from *Aspergillus terreus* cultivation on complex cultivation media. Previously described methods involve extraction with acidified ethyl acetate, which is time consuming and might be a source of analytical errors. As the concentrations of both substances in the medium usually range from tens to hundreds of μg/ml, extraction as a preconcentration step is not necessary.

Using a chromatographic optimization function (COF) and the computer program COST [7], we determined the volumetric fraction of methanol in 18 m*M* orthophosphoric acid solution to be 77.5% for the optimum separation of Lovastatin and mevinolinic acid (resolution > 1.5). Under these conditions no interference from the components of the media with the analyte metabolites was observed (Fig. 2). The duration of one analysis is less than 10 min.

The effluent from the chromatographic column was monitored with a diode-array spectrophotom-

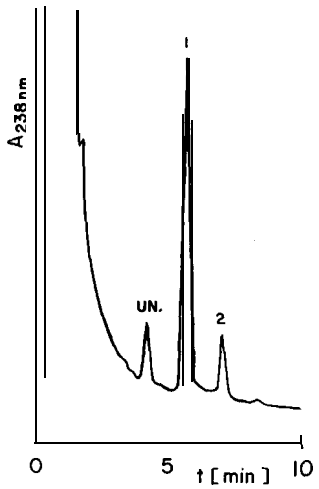

Fig. 2. Chromatogram of fermentation liquid of *Aspergillus terreus* containing (1) mevinolinic acid (424 mg/ml) and (2) Lovastatin (83 mg/ml). UN = unidentified peak. Column, Separon SGX C_{18}; mobile phase, methanol-18 m*M* orthophosphoric acid (77.5:22.5, v/v); flow-rate, 0.5 ml/min; UV detection at 238 nm

TABLE I

CALIBRATION DATA FOR MEVINOLINIC ACID AND LOVASTATIN AT 238 nm

Parameter[a]	Mevinolic acid	Lovastatin
b	$1.18.\ 10^{-3}$	$1.26 \cdot 10^{-3}$
a	$1.28\ 10^{-2}$	$1.49 \cdot 10^{-2}$
r	0.9896	0.9973
D.L.	0.022	0.020

[a] *b*, *a* = Slope and intercept of the calibration line, respectively; *r* = correlation coefficient; D.L. = detection limit @g/ml) for signal-to-noise ratio = 2.

eter. Continuous measurement enables the identity of the peaks to be checked not only according to correspondence of retention times but also by comparison of their UV absorption spectra. Multichannel recording can be also used for checking the peak homogeneity.

Lovastatin and mevinolinic acid display coincidence in their spectra with absorption maxima at 231, 238 and 245 nm, which fit well with published data (23 1,238 and 247 nm) [8]. Calibration data for 238 nm are presented in Table I. The detection limits of Lovastatin and mevinolinic acid (signal-to-noise ratio = 2) under the given conditions are 20-30 ng/ml. The relative deviation for five parallel determinations in fermentation fluid does not exceed 3%. The range of linearity for the determination of Lovastatin and mevinolinic acid was l-500 μg/ml. The day-to-day reproducibility of Lovastatin determination (difference of the average result for the same sample) did not exceed 2% relative.

The proposed method is suitable for the efficient screening of strains for Lovastatin production and possibly also for the determination of Lovastatin and mevinolinic acid in complex biological fluids.

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