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Short Communication

High-performance liquid chromatographic determination of mycophenolic acid in fermentation broth

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ABSTRACT

A precise and specific assay has been developed for the determination of mycophenolic acid in fermentation broth. The method requires little sample preparation and is sufficiently rugged for the analysis of many samples.

INTRODUCTION

Mycophenolic acid is an antibiotic isolated from cultures of *Penicillium* [1–3] that has been demonstrated to possess antiviral as well as antifungal properties. Several methods have been published for the determination of mycophenolic acid including turbidimetric [4], thin-layer chromatographic [5] and gas chromatographic [6] assays. These assays involve relatively elaborate sample preparation and long analysis times. A simple, rapid analysis for mycophenolic acid was needed to monitor the production of mycophenolic acid during the course of fermentation. The method described herein is suitable for the determination of mycophenolic acid in fermentation broth.

EXPERIMENTAL

Chemicals

All solvents and chemicals were of high-performance liquid chromatography (HPLC) grade and used without further purification. Mycophenolic acid was purchased from Aldrich (Milwaukee, WI, U.S.A.).

Apparatus

A Vista 5500 liquid chromatograph and Model 9090 autosampler (Varian Assoc., Palo Alto, CA, U.S.A.) were used. The variable-wavelength detector was set at

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304 nm. The injection volume was 20 μl. Data were collected and analyzed by a Hewlett-Packard HP1000 LIMS system. Unless otherwise specified, DuPont Zorbax Reliance cyano-bonded phase guard and analytical columns were used. The analytical column had the dimensions 80 × 4.0 mm and a particle size of 5 μm. The guard column consisted of the same packing and had the dimensions 10 × 4.0 mm. The mobile phase flow-rate was set at 2 ml/min. All experiments were performed at ambient temperature.

Mobile phase

The aqueous component of the mobile phase was prepared by dissolving 1.5 g of monobasic sodium phosphate in 1 l of HPLC-grade water. The pH was then adjusted to a value of 3.0 with concentrated phosphoric acid and filtered to 0.45 μm. The mobile phase consisted of acetonitrile–buffer (25:75, v/v).

Sample preparation

A 5-ml portion of fermentation broth was extracted with 25 ml methanol. The suspension was placed in an ultrasonic bath for about 5 min and shaken thoroughly. The suspension was then centrifuged in order to facilitate the setting of large particulates. The supernatant was sampled directly and filtered to 0.45 μm prior to injection in the chromatograph. Samples with greater than 500 ppm mycophenolic acid required an appropriate dilution in methanol for quantitative analysis.

RESULTS

Shown in Fig. 1 is a typical chromatogram of mycophenolic acid in fermentation broth extract. The wavelength used is sufficiently selective to allow a rapid assay, since no late-eluting peaks were observed. A cyano-bonded phase was selected over ODS or

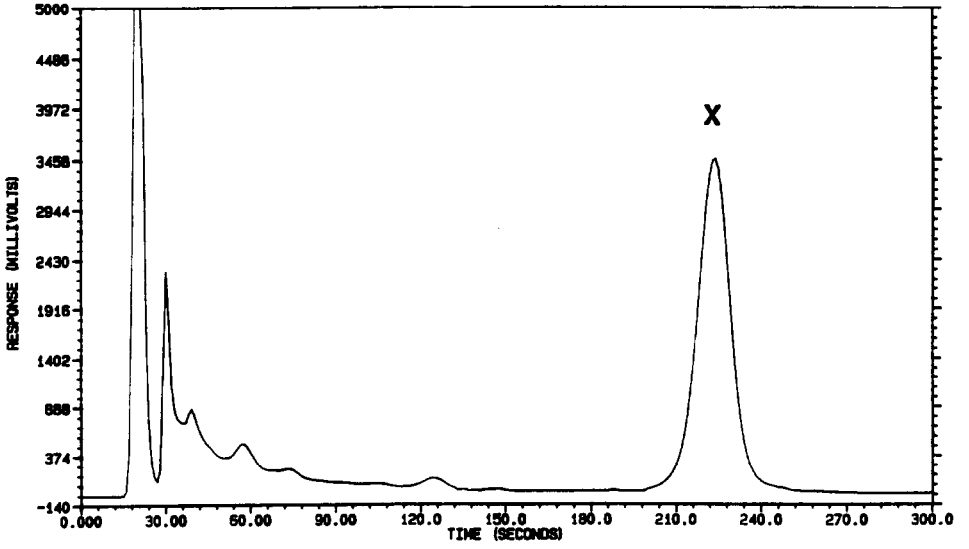

Fig. 1. Chromatogram of 102.6 ppm mycophenolic acid in fermentation extract (analyte peak is marked "X"). Chromatographic conditions are given in the text.

C_8 on the basis of peak symmetry. The alkyl bonded phase produced severely tailed peaks. Under the chromatographic conditions described, the values of the asymmetry and theoretical plate count were 1.01 and 17 500 theoretical plates per meter, respectively. A test of precision with a 102.6-ppm sample ($n = 11$) gave a relative standard deviation of less than 1%. The method was linear over the domain 5 to 500 ppm (correlation coefficient = 0.999) using peak area for quantitation. The upper limit of the linear response using peak height was 300 ppm.

To evaluate the possibility of matrix effects on the assay, a series of standards ranging from 5 to 500 ppm in mycophenolic acid was prepared in a methanol extract of a fermentation broth. The broth was sampled early in the course of fermentation to minimize the concentration of mycophenolic acid. The slope of a plot of peak area *versus* concentration for this standard series was 30.4 arbitrary units. The slope of a calibration plot of standards prepared in methanol was 31.7 units, only a 4% difference. In addition the intercept of the former calibration plot occurred at the origin. Therefore, no matrix interferences were observed.

The extraction efficiency was evaluated by spiking a known volume of fermentation broth with solid mycophenolic acid. The suspension was mixed well prior to the addition of extraction solvent to simulate an actual sample. Over the concentration domain 0.2 to 4.0 mg/ml, the recovery of analyte averaged 98.9% with a standard deviation of 3.3% ($n = 14$), indicating complete extraction of the mycophenolic acid from the broth.

The durability of the method was assessed by repeatedly injecting a solution of broth extract. After analyzing 100 samples of fermentation broth, the theoretical plate count and retention time remained constant, indicating the lack of accumulation of matrix components on the column. The coefficient of variation of the peak area was 0.7%.

CONCLUSIONS

A rapid assay for the determination of mycophenolic acid in fermentation broth has been described. The assay is more precise than published methods and requires less sample preparation. On the basis of a signal-to-noise ratio of 5, the detection limit is about 20 ng. The linear dynamic range of the method is particularly useful in monitoring in-process fermentation.

REFERENCES

1 K. Ando, S. Suzuki, G. Tamura and K. Arima, *J. Antibiotics*, 21 (1968) 649.
2 R. H. Williams, D. H. Lively, D. C. DeLong, J. C. Cline, M. J. Sweeney, G. A. Poore and S. H. Larsen, *J. Antibiotics*, 21 (1968) 463.
3 S. Suzuki, T. Kimura, K. Ando, M. Sawada and G. Tamura, *J. Antibiotics*, 22 (1969) 297.
4 F. E. Gainer and R. L. Hussey, *J. Chromatogr.*, 54 (1971) 446.
5 T. Noto, Y. Harada and K. Koyama, *J. Antibiotics*, 23 (1970) 96.
6 F. E. Gainer and H. J. Wesselman, *J. Pharm. Sci.*, 59 (1970) 1157.