Analysis of Amphotericin B by Micellar Electrokinetic Capillary Chromatography with Scanning Detection

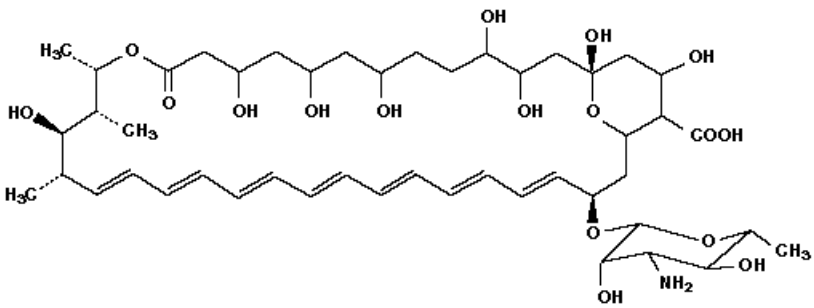

Fig. 1. Structure of amphotericin B.

Analysis Conditions

Instrument	BioFocus® 3000 system
Polarity	+ to -, negative at the detector end
Capillary	50 µm x 50 cm, uncoated (catalog number 148-3040)
Run buffer	10% Basic Protein Analysis Buffer (catalog number 148-5023), 10% acetonitrile, 80% deionized water with 50 mM sodium dodecyl sulfate
Injection	20 psi * sec
Run voltage	10 kV
Detection	scanning, 375-450 nm
Cartridge temperature	20 °C
Autosampler temperature	20 °C

Introduction

Amphotericin B is a potent drug used in the treatment of fungal infections. The polyene-type antibiotic (Figure 1) is obtained from a strain of *Streptomyces nodosus*. In this note, micellar electrokinetic capillary chromatography was carried out using capillary electrophoresis instrumentation, and scanning detection in the visible range was used to confirm peak identity.

Results

Micellar electrokinetic capillary chromatography (MECC) is a mode of CE which is suitable for a wide variety of charged or neutral substances. Surfactants such as sodium dodecylsulfate (SDS) are added to the run buffer and the separation mechanism involves partitioning of the analyte between a pseudo-stationary phase and a mobile phase. Often an organic solvent is added to the buffer to increase solubility of the analytes or to change the separation selectivity. A run buffer with 10% acetonitrile and 50 mM SDS was used in the MECC separation shown in Figure 2. Sample preparation included use of 10% DMSO to dissolve the antibiotic. Figure 3 shows the spectrum acquired at 50.7 minutes as the sample zone migrated through the detection point. Two distinct maxima, at 390 and 410 nm, can be distinguished.

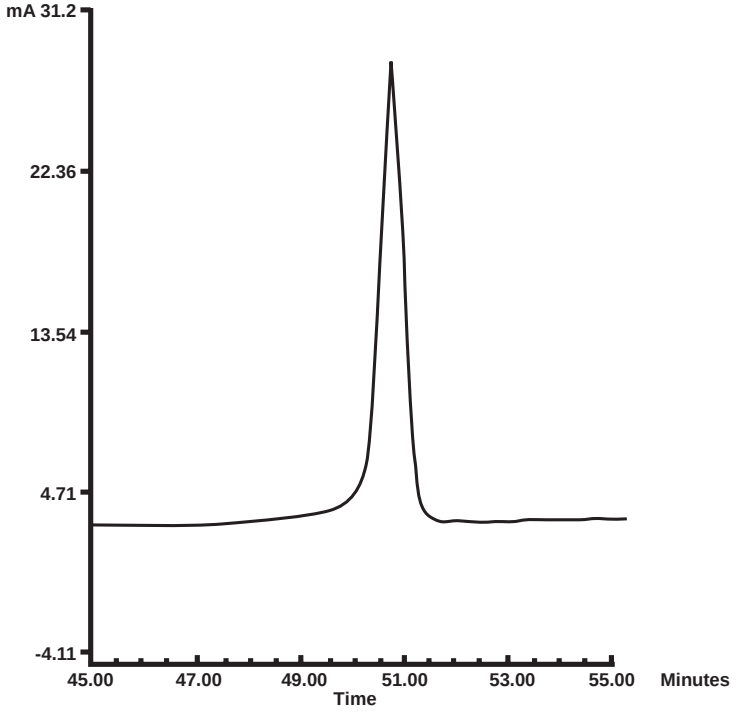

Fig. 2. MECC separation of amphotericin B with detection at 410 nm.

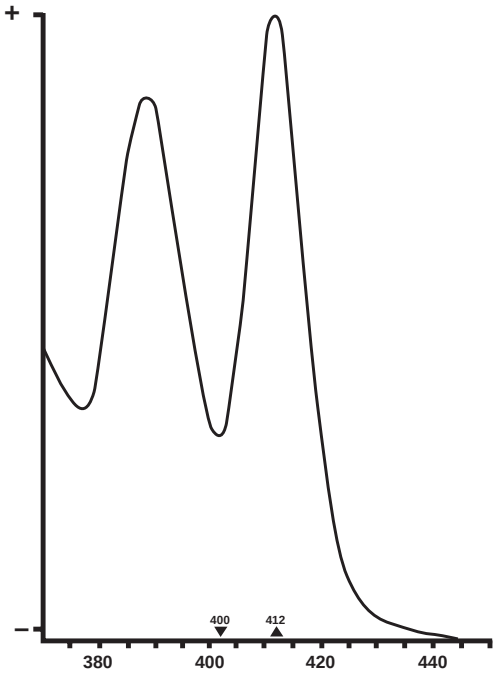

Fig. 3. Visible spectrum (375-410 nm) of the peak migrating past the detection point at 50.7 min.

Bio-Rad Laboratories

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Bio-Rad Laboratories Main Office, *2000 Alfred Nobel Drive, Hercules, California 94547, Ph. (510) 741-1000, Fx. (510) 741-1060* •
Eastern Regional Office, *85A Marcus Dr., Melville, New York 11747, Ph. (516) 756-2575, Fx. (516) 756-2594* •
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