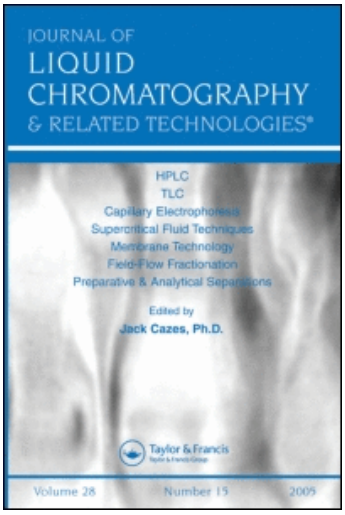

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PREPARATIVE LIQUID CHROMATOGRAPHY OF PHARMACEUTICALS USING SILICA GEL WITH AQUEOUS ELUENTS

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ABSTRACT

Acidic, basic, and neutral pharmaceuticals can be easily assayed on silica with aqueous eluents. Because of the nature of these packings, chromatographic methods are more reproducible than bonded phase packings and can be easily scaled up for preparative procedures.

INTRODUCTION

In the pharmaceutical industry where a vast majority of products are either organic acids or bases, asymmetric peaks or non-elution of components have been common observations; especially when developing methods based on silica gel using non-aqueous eluents. Column-to-column and day-to-day variations when using silica have also been noted. Furthermore, recoverable yields from preparative silica columns are rarely quantitative (1).

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Bad chromatographic performance can be due to numerous causes with the interaction of eluate with silanol groups being the most commonly cited reason. Until recently has it been shown that the relatively large amounts (0.3% by weight) of iron and other metal oxide impurities detected in silica gel appear to be the major contributors to poor chromatographic performance (2-6). Fortunately, these metal impurities can be washed from silica gel with dilute acid (3,4,7) which, consequently, improves chromatographic performance; particularly the recoverable yield from preparative columns (3). In addition to washing the silica gel with acid, we have found that with both preparative and analytical modes the use of aqueous eluents has further increased the reliability of silica gel chromatographic systems (8).

The utility of silica gel columns, especially when using aqueous eluents for pharmaceutical analysis, has been demonstrated in the following citations: tricyclic antidepressants (9-10), 151 basic pharmaceuticals (11-12), nucleosides (13), amino acids (14), sulfonamides (14), anticholinergics (14), biogenic amines (14-15), organic acids (16), dipeptides (17), antihistamines (18-19), carbohydrates (20-22), carotenes (23), barbiturates (10), the β-blockers propranolol (24) and nadolol (8), sulfadiazine (25) and drugs of forensic interest (26).

This article highlights the authors' approach to preparative liquid chromatography of the two pharmaceuticals aztreonam and nystatin using silica gel with aqueous eluents in isolating and identifying impurities found in drug substances.

MATERIALS AND METHODS

Preparative procedures

A Waters Associates 500A high-performance liquid chromatograph with a Prep-500 silica column was used. After removal of any organic solvents, preparative fractions were either lyophilized or concentrated on a C-18 solid phase extractor (Sep-Pak®, Waters).

Semi-preparative and analytical procedures

The HPLC system consisted of a model 110A pump (Beckman) and a Waters Z-module with a 10cm x 8mm i.d. column packed with 5-μm silica or a Waters semi-prep instrument with a 15cm x 19mm i.d. column packed with 5-μm silica. The flow rate was 3-5 mL/min. Detection was by means of a model 773 UV detector (Kratos). Sample injections of 200 μL were made using a WISP® 710B autoinjector (Waters). All other commercially available silica columns should be thoroughly washed with isopropanol prior to the acid wash (1% phosphoric acid) and equilibration with aqueous eluents.

Mass spectrometry procedures

Fast atom bombardment mass spectra were obtained by sputtering (8 KeV Xe) a thioglycerol or dithiothreitol-dithioerythritol (ca. 5:1) solution of the preparative isolate. The resulting secondary ions were analyzed using a VG-ZAB-2F mass spectrometer. MS/MS spectra were obtained by mass analyzing the appropriate parent, exciting this ionic species by collision with nitrogen, and energy analyzing the resulting fragments. Accurate mass measurements were

accomplished by introducing an approximately equal volume of a 1% aqueous solution of polyethylene glycol as an internal standard.

Sample

Bulk powders of aztreonam and Chinese nystatin were obtained from E.R. Squibb and Sons. Samples were dissolved in the mobile phase.

Mobile Phase

A eluent composition of 0.1% phosphoric acid was used for the preparative isolation of aztreonam. For nystatin, a mobile phase of acetonitrile-aqueous (1 mM) ammonium dihydrogen phosphate (13:87) was utilized.

RESULTS AND DISCUSSION

During the past several years, the FDA has increased the emphasis on identifying and limiting impurities found in both drug formulations and bulk drug substances (27) which naturally has also increased development of preparative procedures.

We have found that the one major advantage of developing preparative methods on silica gel chromatographic columns, particularly after acid washing the column, is the ability to directly translate an analytical method to the preparative mode without experiencing differences in retention selectivity. Variations in retention selectivity are common among different reversed-phase columns (28).

Aztreonam

In an aqueous solution, one of the most important sources of instability of aztreonam over the whole pH range would be expected to be hydrolysis of the beta-lactam ring. An analytical chromatogram of a temperature-stressed aqueous solution of aztreonam indicated that at least two degradants were formed (Fig. 1). A direct scale up was successfully performed using a Waters System 500A chromatograph. The separately collected degradants were concentrated directly onto C-18 solid phase extractors (Sep Pak®), washed with water, and eluted with methanol. The methanol fractions were dried under a stream of nitrogen. The two dried fractions were reconstituted in methanol and analyzed by positive and negative ion FAB as well as MS/MS characterization.

The degradant eluting prior to aztreonam (R_T 2 min.) exhibits the protonated and deprotonated molecule characteristic of the hydrolyzed product of aztreonam. These parent ions are evident at m/z 454 and 452, respectively. Details of the fragmentation as determined from their MS/MS spectra are shown in Figure 2.

For the degradation peak eluting after aztreonam (R_T 8.5 min.), the parent ions at m/z 434, $(M-H)^-$, and m/z 436, $(M+H)^+$, appeared identical to those of aztreonam even under high resolution conditions. Examination by MS/MS methods (Figure 3) of both aztreonam and this degradant shows differences in the relative ion intensity of the 354^- fragment (SO_3 loss) to the 348^- fragment ($C_4H_6O_2$ loss). As desulfonation occurs remote to any expected isomeric site, it is less likely to be influenced by an isomeric difference than is the loss of the side chain ($C_4H_6O_2$). Loss of

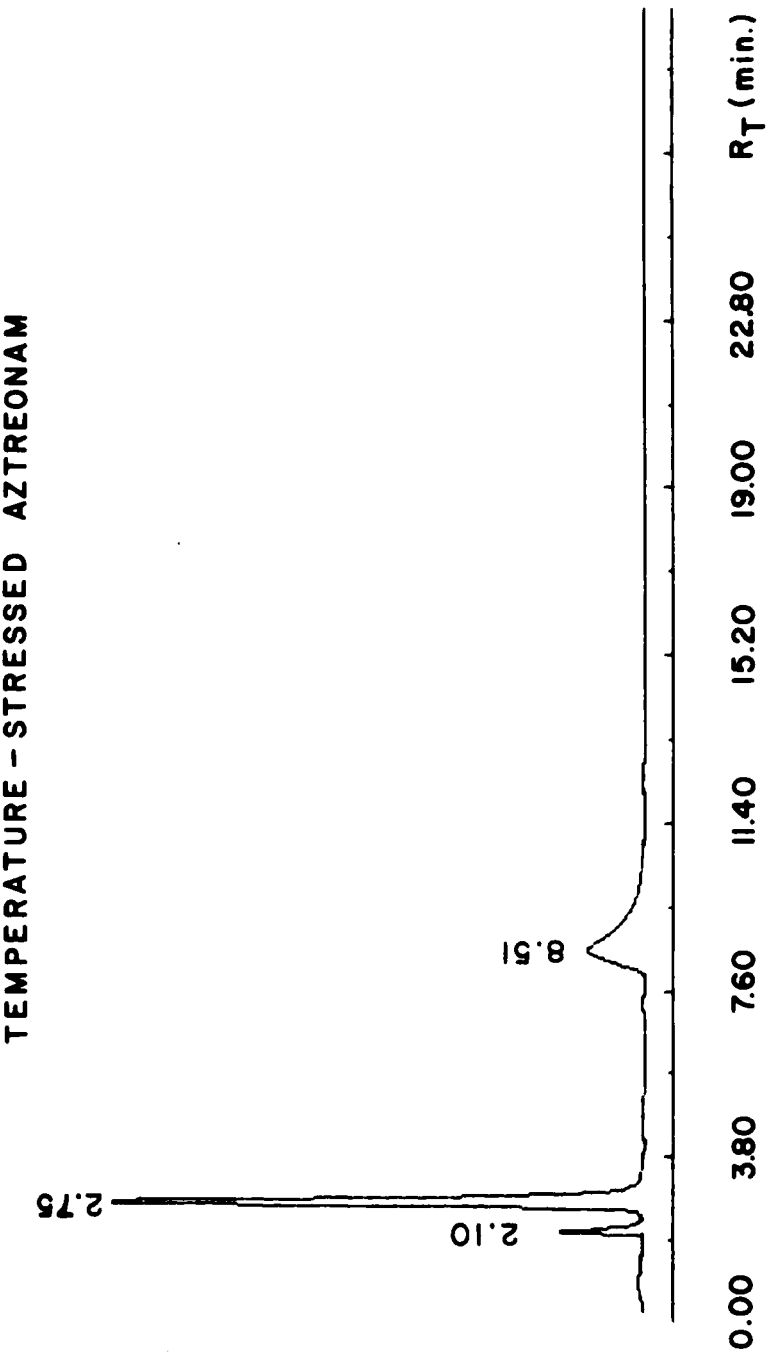

Figure 1. Chromatogram of degraded aztreonam.

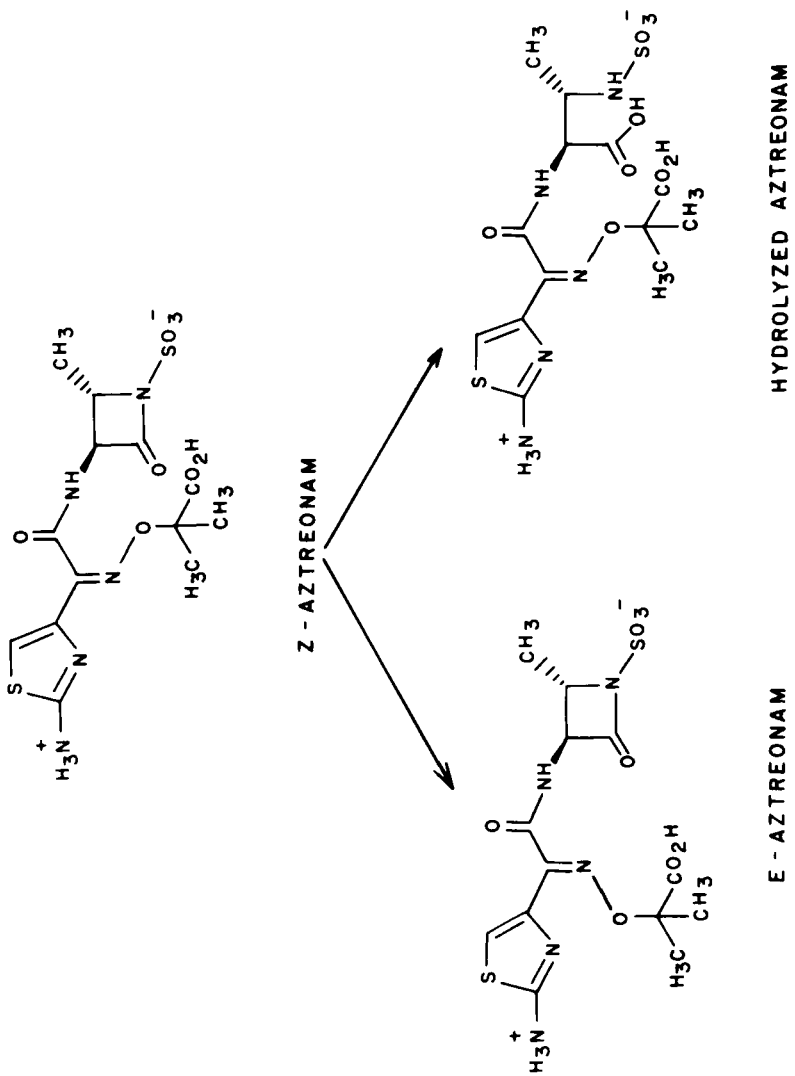

Figure 2. Fragmentation scheme of the hydrolysis degradant of aztreonam.

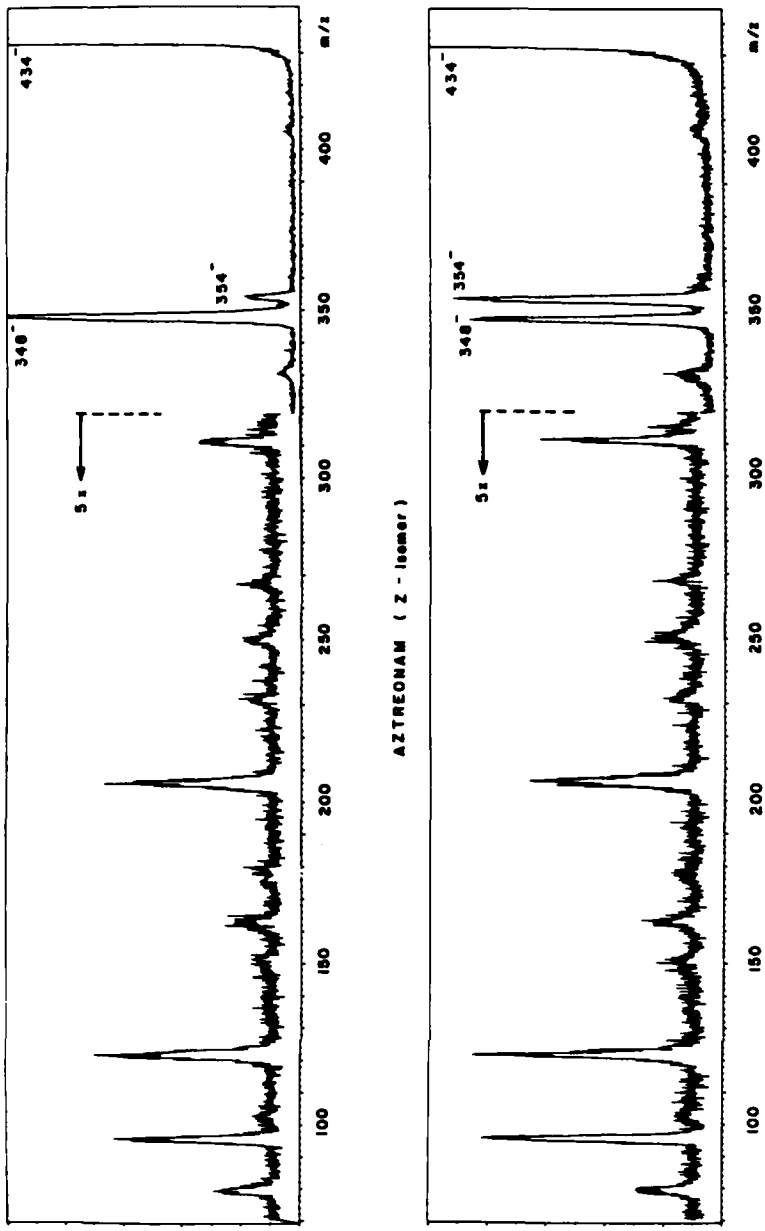

Figure 3. Comparison of the FAB MS/MS spectra of the deprotonated molecule of an HPLC isolate (top) with that of authentic aztreonam (bottom).

the side chain is influenced most by differences between the E- and Z-forms of aztreonam (29). The suggested presence of the E-isomer of aztreonam was confirmed by spectral comparisons with an authentic sample.

In conclusion, under these conditions the two major pathways of aztreonam degradation are hydrolysis and isomerization (Fig. 4). It is interesting to note that either degradation product is inactive as an antibiotic.

Chinese Nystatin

Four major components are present in Chinese Nystatin (Fig. 5). The preparative procedure was scaled up to a 10cm x 8mm silica column. The collected fractions were handled in a similar manner to that of aztreonam degradants except that the acetonitrile in these preparative fractions was blown off with a stream of nitrogen prior to solid phase extraction. Mass spectral analysis of each component established their molecular weights and formulae (from accurate mass measurement). Analysis using MS/MS methods indicated the presence of either one or two sugars (mycosamine or digitoxose). Figure 6 illustrates the proposed structures for the four major components of Chinese nystatin as evident from their mass spectra. Three of the four are known, Nystatin A_1, Nystatin A_3, and Polyfungin B. Deoxynystatin A_1 is suggested on the basis of its molecular weight and formula, its apparent fragmentation as observed in the MS/MS spectrum, and by its relationship to Polyfungin B and Nystatin A_3.

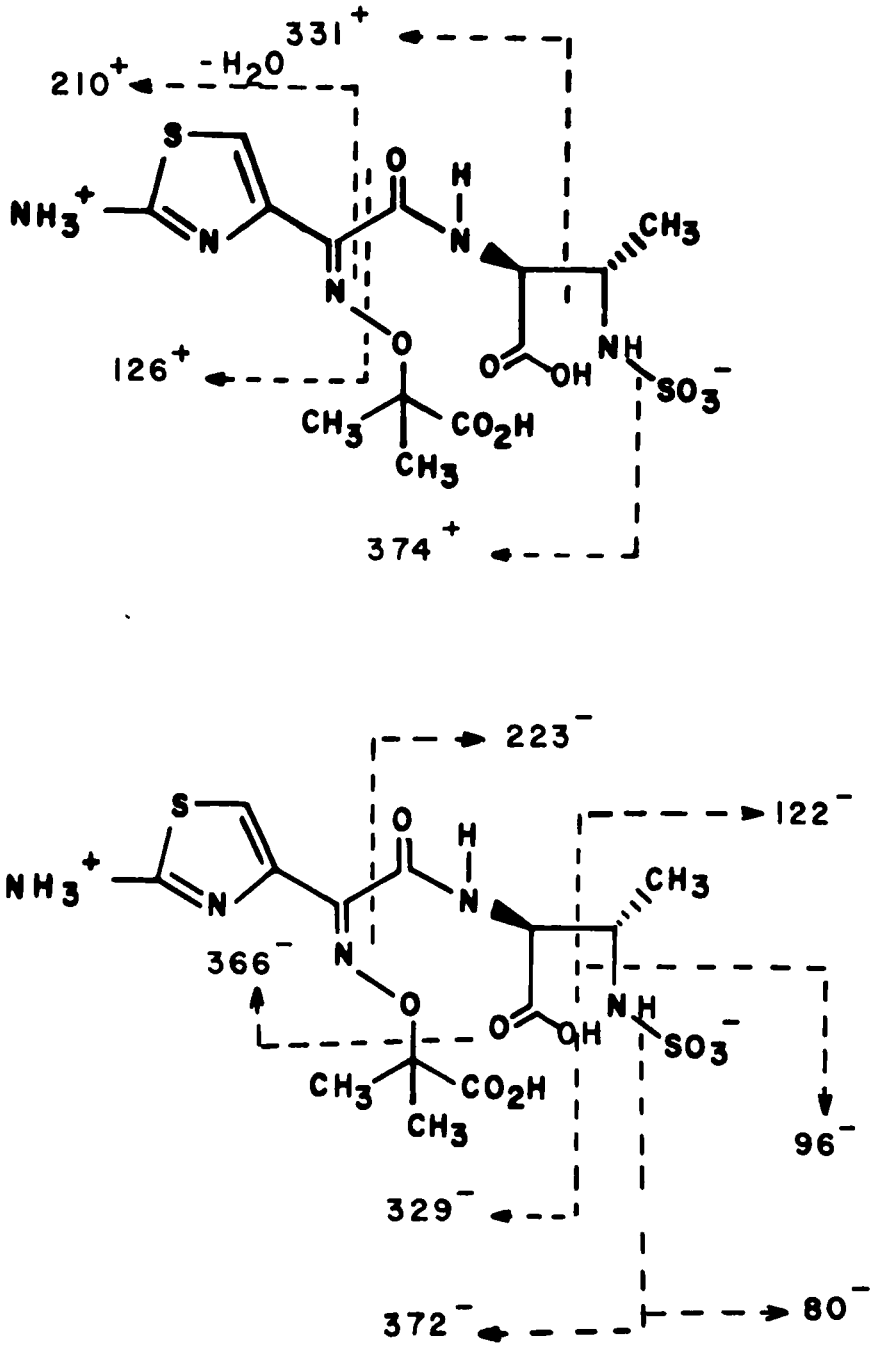

Figure 4. Degradation pathway of aztreonam.

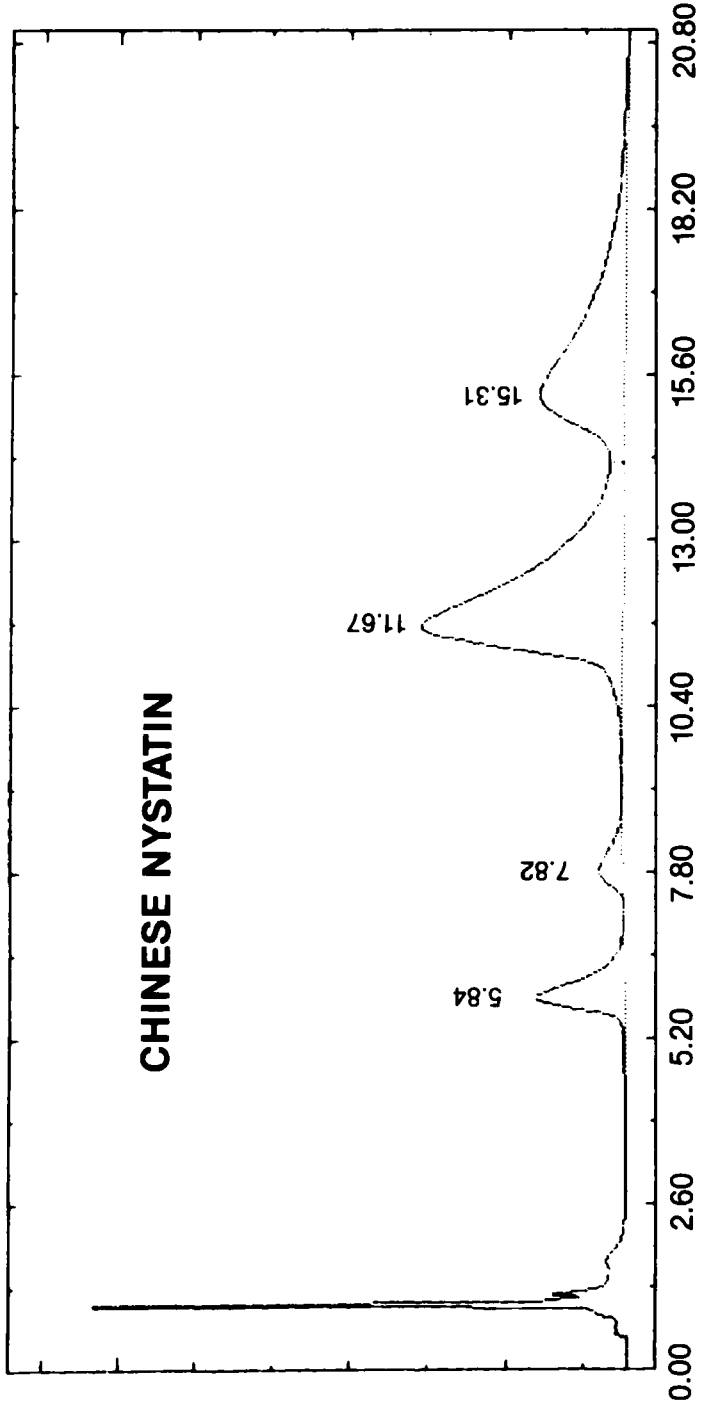

Figure 5. Chromatographic profile of Chinese nystatin.

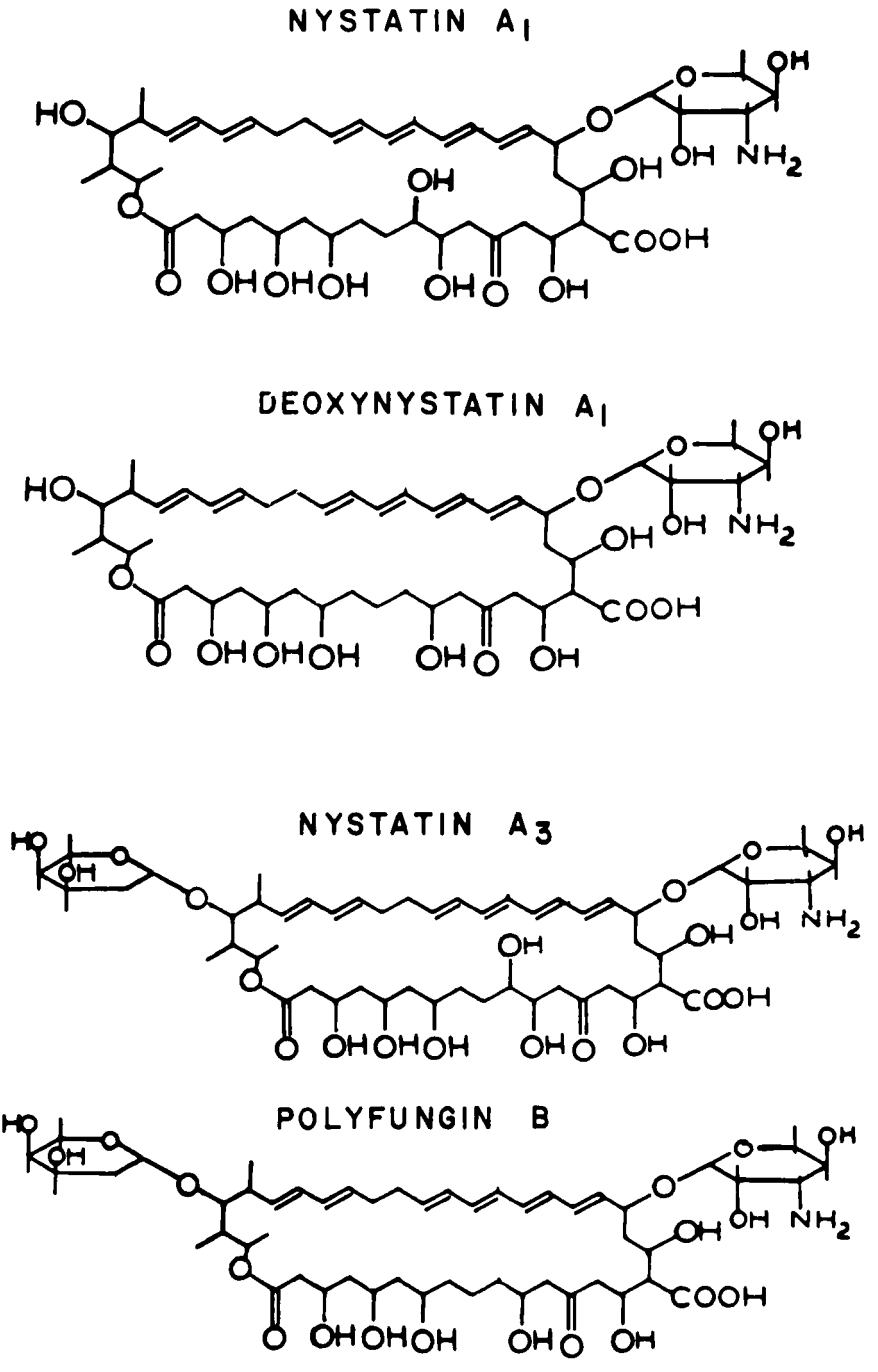

Figure 6. Constituents of Chinese nystatin.

These are but two examples of where we have successfully scaled up an analytical aqueous silica method to a preparative method. There are few literature citations where preparative silica gel methods using aqueous eluent have been reported (30-31).

Systematic approaches to developing chromatographic methods using silica with aqueous eluents have been documented (8,13,19,24,26). The dominant separation mechanism appears to be due to the ion-exchange capacity of silica (19). Additional substance-eluent-interactions of silica in polar eluents have also been demonstrated (32).

Besides the ease of developing preparative methods based on silica and aqueous eluents, bulk silica is cheaper than reversed-phase packings. Unfortunately, the perception that reversed-phase chromatographic columns are better suited for solving chromatographic problems than silica columns is a commonly held belief that goes hand in hand with the chromatographic literature citations of residual silanol groups on chemically bonded columns being the universal scapegoats for poor peak shapes.

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