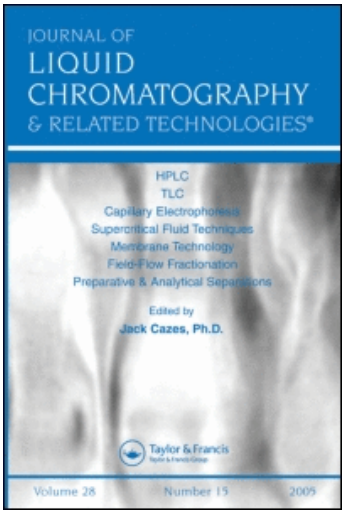

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SOLID PHASE EXTRACTION OF SULFONAMIDES USING CYCLOBOND-I CARTRIDGES

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The use of cyclobond-I solid phase extraction (SPE) cartridges in the analysis of sulfonamides was investigated. An aqueous solution of sulfonamides in 0.1 M potassium phosphate buffer (pH 4.0) was passed through the SPE cartridge. The sulfonamides which were retained on the cartridge by formation of inclusion complexes between the sulfonamides and B-cyclodextrin were eluted with 50% aqueous methanol. The eluate was directly analysed by High Performance Liquid Chromatograph with UV detection at 265nm.

INTRODUCTION

Solid phase extraction (SPE) technique, as a sample clean-up tool, has gained wider acceptance in recent years. Several silica based sorbents are commercially available and

are being used for isolation of a variety of chemical compounds from biological and nonbiological matrices. Cyclobond-I solid phase extraction cartridges, which contain B-cyclodextrin bonded to silica, have been used for certain types of compounds (1, 2).

In this study, I have explored the use of cyclobond-I SPE cartridges for the selective extraction of sulfonamides from aqueous medium.

EXPERIMENTAL

Reagents and standards:

a) Chemicals.- Sulfanilamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethiazole, and sulfamethazine (Sigma Chemical Co., St. Louis, MO). Potassium phosphate (mono and dibasic), ammonium acetate, acetic acid and HPLC grade methanol (Fisher Chemical Co., Fairlawn, NJ).

b) Potassium phosphate buffers.- 0.1M potassium phosphate buffers at pH 4.0, 4.5, 5.0, 5.5, and 6.0 were prepared with monobasic potassium phosphate and the pH was adjusted with either dibasic potassium phosphate or with phosphoric acid. Similarly, 0.2 to 1.0M buffers were prepared with monobasic potassium phosphate and the pH adjusted to 4.0 with phosphoric acid.

c) Mobile phase for HPLC.- 0.25 M ammonium acetate was prepared by dissolving 1.92 gm ammonium acetate in 900 ml distilled water, the pH was adjusted to 4.7 with acetic acid and the volume made to 1000 ml with distilled water. The

mobile phase was prepared by mixing 880 ml buffer with 120 ml methanol.

d) Standard solution of sulfonamides.- Sulfanilamide, sulfadiazine, sulfathiazole, sulfapyridine, sulfamerazine, sulfamethiazole and sulfamethazine (100 mg each) were dissolved in 100 ml methanol. A one ml portion of this solution was diluted to 100 ml with distilled water (solution B).

e) Working Standard.- Ten ml of solution B was diluted to 100 ml with distilled water to provide a solution with a concentration of each sulfa drug at 1000 ng/ml.

Apparatus:

a) Solid phase extraction cartridges.- Cyclobond-I, SPE columns, 3 ml size (Advanced Separation Technologies Inc., Whippany, NJ).

b) Liquid Chromatograph.- A constametric III solvent delivery pump equipped with a 7125 Rheodyne injector and a SpectroMonitor III variable wavelength uv detector was used (LDC/Milton Roy, Riviera Beach, FL). An HP 3390 integrator was used for quantification.

c) HPLC Column.- LC-DB-18, 25 cm x 4.6 mm, 5um particle size (Supelco Inc., Bellefonte, PA).

Procedure:

The cyclobond-I SPE column was first washed with 5 ml distilled water followed by 5 ml potassium phosphate buffer. A solution of 200 ng of each of the seven sulfonamides in 5

ml buffer was passed through the SPE cartridge. Sulfonamides, which were retained on the cartridge, were eluted with 3 ml aqueous (50%) methanol. The total eluate volume was diluted to 4 ml with aqueous methanol and 50 ul was injected into the HPLC.

Liquid Chromatography:

The mobile phase flow rate was set at 1.3 ml/minute. The SpectroMonitor was set at 265 nm wavelength with sensitivity at 0.01 AUFS. Quantification was done by peak heights as measured by the HP3390 integrator.

RESULTS AND DISCUSSION

Figure 1 shows a representative chromatogram of seven sulfonamide standards. The percent retention of sulfonamides on the SPE cartridge was determined by HPLC analysis.

Cyclobond-I SPE cartridges contain cyclodextrins bonded to silica. Cyclodextrins are D (+) glucopyranose units connected by α-(1,4) bonds to form cyclic oligosaccharides (3). Three commercially available cyclobond cartridges, cyclobond-I, II, and III contain B, α and γ cyclodextrin, respectively, and differ in structure by the number of glucose units present (3). Cyclodextrinα, B,$\gamma$and contain 6, 7, and 8 glucose units, respectively. These glucose units are arranged in a fashion to form a truncated cone shape. The basic difference among the three cyclodextrins is the size of the cone. The orientation of

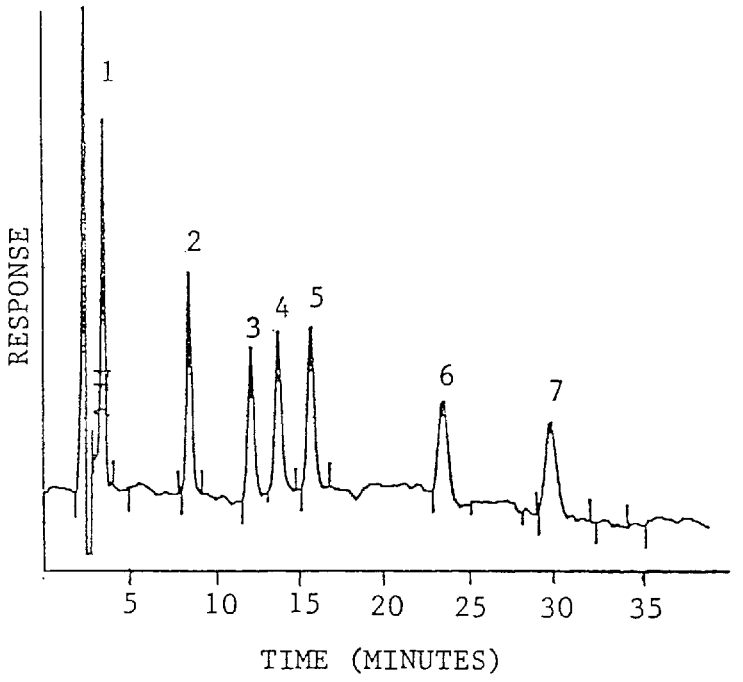

Figure 1. Chromatogram of seven standard sulfonamides. 1. Sulfanilamide, 2. Sulfadiazine, 3. Sulfathiazole, 4. Sulfapyridine, 5. Sulfamerazine, 6. Sulfamethiazole, 7.Sulfamethazine.

glucose units is such that there are no hydroxyl groups on the interior of the cavity making it hydrophobic (4).

The formation of an inclusion complex of B-cyclodextrin with various drugs including N-phenylanthranilic acids, phenothiazines, 4-barbiturates, cinnamates, prostoglandins and sulfonamides in aqueous solutions have been reported (5-9). Sulfonamides can form a stable inclusion complex with B-cyclodextrin, but very

little or no inclusion complex formation was observed when α-cyclodextrin was used (10,11). This is attributed to the smaller cavity size of α-cyclodextrins.

The aim of this work was to find the optimum conditions for the formation of the inclusion complex between sulfonamides and B-cyclodextrin, and to take advantage of this for the selective isolation of sulfonamides from various biological matrices.

Since sulfonamides are ionic in nature, their retention on the cyclobond-I SPE cartridges was pH dependent. The pH profile for the formation constant of sulfathiazole-B-cyclodextrin complex has been studied, and maximum interaction for formation of a complex was observed around pH 5 to 5.5 (10). This suggested that the maximum retention of sulfathiazole on cyclobond-I SPE cartridge can be achieved in this pH range. In order to determine the optimum retention conditions of other sulfonamides, five 0.1M potassium phosphate buffers (pH 4.0, 4.5, 5.0, 5.5, and 6.0) were prepared and used as described under methods. No recoveries were obtained for sulfanilamide, suggesting no retention of this drug on the cartridge. The recoveries of sulfadiazine, sulfathiazole, sulfapyridine and sulfamerazine were over 90% in all cases. Maximum recovery (87%) of sulfamethazine was obtained within a pH range of 5.0 to 5.5, which dropped to 80% when the pH was increased to 6.0. The effect of pH was more pronounced for sulfamethiazole and maximum recovery (85%) was obtained at pH 4.0. Increasing

the pH resulted in a drop of recovery to almost no recovery at pH 5.5 and above.

The effect of molar strength of buffer had some effect on the retention of different sulfonamides, but was not very significant. Potassium phosphate buffers with molar strengths of 0.1, 0.2, 0.5, and 1.0 were tested and a slight increase in the retention of sulfonamides was observed with increasing molar concentration of the buffer. The most noticeable difference was observed in the case of sulfamethazine, where recoveries were increased from 87% to 94% when the concentration of buffer was increased from 0.1M to 1.0M.

The recoveries of sulfonamides were also decreased when the cartridges were washed with water. This was probably due to the change in pH which would have affected the retention of sulfonamides on the cartridges. Washing the cartridges with the same buffer as used to load the sulfonamides did not reduce recoveries.

The elution of sulfonamides from the SPE cartridges was possible, either by changing the pH of the elution buffer or using an organic solvent such as methanol. When 0.1M (pH 10.0) potassium phosphate buffer was used as an eluting buffer, incomplete elution of sulfonamides was observed. Also, silica may be attacked at this pH. Therefore, this elution buffer was not found suitable. The second choice was to use methanol, which gave complete elution of sulfonamides. Once the sulfonamides were eluted, however, it was necessary to evaporate the eluate to dryness

and redissolve eluted sulfonamides in water in order to obtain good resolution on HPLC. When the elution buffer was changed to 50% aqueous methanol, more elution volume (2 to 3 ml as compared to 1.5 to 2.0 ml) was required for complete elution of all the sulfonamides. This eluate, however, was directly injected into HPLC without any loss in peak resolution. Therefore, a 50% aqueous methanol was chosen as the eluting solvent.

The use of cyclobond-I SPE cartridges in the analysis of sulfamethazine in milk has successfully been demonstrated (12). Work is in progress for a multiresidue method for the analysis of sulfonamides in milk.

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