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COMMUNICATIONS

EFFECT OF MOBILE PHASE pH ON THE SEPARATION OF DRUGS USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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ABSTRACT

When developing a HPLC assay method for drugs, the pH of the mobile phase can be effectively used as a tool to accomplish complete separation. Using two weak acids (hydrocortisone sodium succinate and methylprednisolone sodium succinate) and a weak base (promethazine hydrochloride), the changes in the chromatograms (retention times and separation of peaks) have been determined at 7 different pH values of a mobile phase with other conditions remaining the same. It has been shown that the retention times of weak acids will decrease with increasing pH values while those of weak bases will increase with increasing pH. The optimum pH value for the separation of these drugs has been determined to be 5.

INTRODUCTION

The separation of drugs in multicomponent dosage forms is often a challenge to a pharmaceutical analyst. Majority of the drugs are either weak bases or weak acids. To separate either weak bases, weak acids, or weak bases from weak acids, the pH of the mobile phase is an important consideration. Often by changing the pH, these separations can be made.

Mobile phases containing a constant organic solvent strength in water, but whose pH values have been changed, will usually change the retention time of weak acids or weak bases. If the pH is increased, the weak bases will have longer retention times while the weak acids will have shorter retention times.

The purpose of these investigations was to demonstrate the effect of pH on the separation and retention times of 2 weak acids and a weak base. Two weak

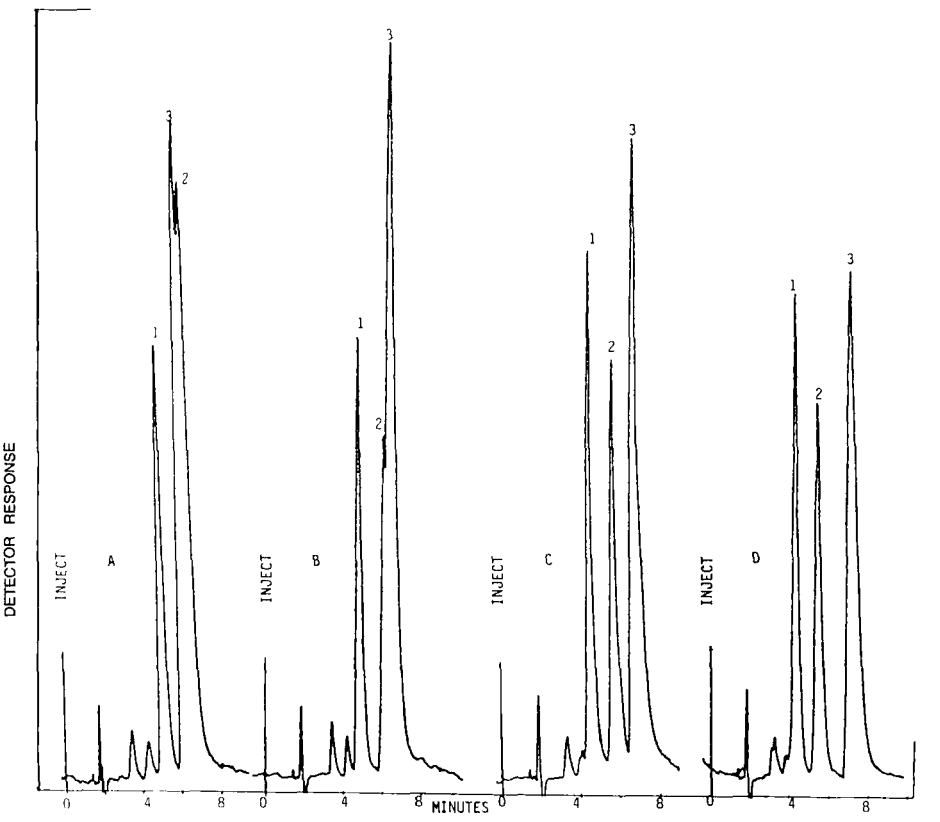

Figure 1 : Sample chromatograms. Peaks 1-3 are from hydrocortisone sodium succinate, methylprednisolone sodium succinate, and promethazine, respectively. Chromatograms A-D were developed using mobile phase of pH 4.0, 4.5, 4.8, and 5.0, respectively. For chromatographic conditions, see text.

acids, hydrocortisone sodium succinate and methylprednisolone sodium succinate and a weak base, promethazine hydrochloride were used in these investigations.

MATERIALS AND METHODS

Chemicals and Reagents: All the chemicals and reagents were either USP-NF or ACS grade and used without further purification. Hydrocortisone sodium succinate powder for injection which also contained benzyl alcohol as the

preservative (Abbott Labs) and methylprednisolone sodium succinate containing benzyl alcohol as preservative (The Upjohn Co.) were from the commercial lots. Promethazine hydrochloride powder, USP was purchased from Napp Chemicals, Inc., Lodi, NJ 07644.

Apparatus: A Waters ALC 202 chromatograph (Waters Associates, Milford, MA) equipped with a universal injector (Rheodyne Model 7125), a multiple wavelength detector (Schoeffel's SF 770, Applied Biosystems) and a recorder (Omniscribe 5213-12, Houston Instruments, TX) was used. A micro C_{18} column (Whatman, 25cm x 4.5mm i.d.) was the stationary phase.

Chromatographic Conditions: The mobile phase contained 40% (v/v) acetonitrile in 0.01 M potassium dihydrogen phosphate in water. The pH of the mobile phase was adjusted using either 1N acetic acid or 1N sodium hydroxide solution. Mobile phases with 7 pH values (4.0, 4.5, 4.8, 5.0, 5.3, 5.6 and 6.0) were prepared for this study. The flow rate was 2.0 ml/min, the wavelength was 254 nm (sensitivity 0.1 AUFS), the chart speed was 30.5 cm/h and the temperature ambient. The injection volume was 20 µl.

Preparation of Stock and Standard Solutions: The stock solutions of hydrocortisone sodium succinate (1.0 mg/ml), methylprednisolone sodium succinate (1.0 mg/ml) and promethazine hydrochloride (1.0 mg/ml) were prepared in water using a simple solution method. The appropriate quantities of the stock solutions were mixed and diluted with water to prepare a standard solution for injection containing 80 µg/ml of hydrocortisone sodium succinate, 80 µg/ml of methylprednisolone sodium succinate, and 50 µg/ml of promethazine hydrochloride.

RESULTS AND DISCUSSION

Chromatogram A (Figure 1) was developed using a mobile phase of pH value 4.0. In this chromatogram, promethazine HCl (peak 3) did not separate from methylprednisolone sodium succinate (peak 2) and its retention time is actually less than methylprednisolone sodium succinate. Since promethazine is a weak base, its retention time decreased at lower pH values while that of methylprednisolone as well as hydrocortisone increased.

Chromatogram B (Figure 1) was developed using a mobile phase of pH value 4.5. In this chromatogram, promethazine HCl (peak 3) eluted out with a longer retention time, but not enough for a complete separation from methylprednisolone sodium succinate.

Chromatogram C (Figure 1) was developed using a mobile phase of pH value 4.8. In this chromatogram, the separation of all the three compounds is almost complete.

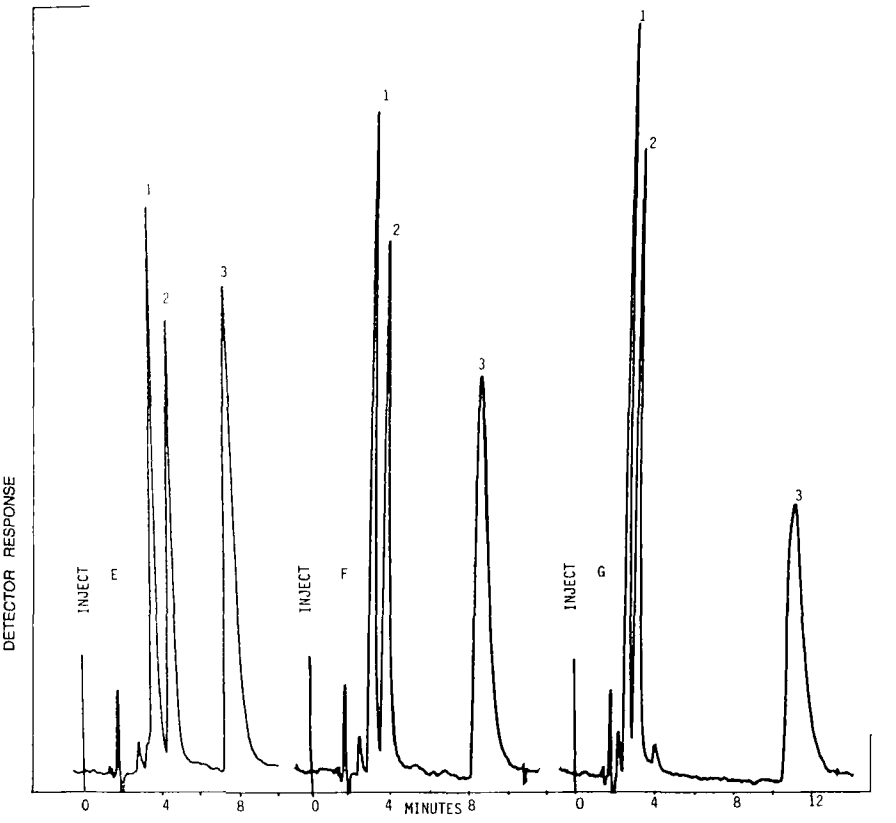

Figure 2 : Sample chromatograms. Peaks 1-3 are from hydrocortisone sodium succinate, methylprednisolone sodium succinate, and promethazine, respectively. Chromatograms E-G were developed using mobile phase of pH 5.3, 5.6, and 6.0, respectively. For chromatographic conditions, see text.

Chromatogram D (Figure 1) was developed using a mobile phase of pH value 5.0. In this chromatogram, the base line separation is very good. This is considered the best pH value for the separation of all the 3 ingredients.

Chromatograms E, F, and G (Figure 2) were developed using mobile phases of pH values 5.3, 5.6 and 6.0, respectively. These were developed to prove that the retention time of a weak base (promethazine) will keep increasing by increasing the pH value of the mobile phase. Those of the weak acids will keep decreasing by increasing the pH value of the mobile phase. In chromatograms F and G, the retention times of the weak acids (methylprednisolone and hydrocortisone) have decreased to such a point that the separation between them became incomplete.

In all the chromatograms, the peaks before the hydrocortisone sodium succinate are from the excipients such as benzyl alcohol which is added as preservative in the commercially available powders for injection of hydrocortisone sodium succinate and methylprednisolone sodium succinate.