SIMULTANEOUS SEPARATION OF PHARMACEUTICAL SUBSTANCES AND COUNTERIONS USING A SERIAL CONNECTION OF HYDROPHILIC INTERACTION AND REVERSED PHASE COLUMNS

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Abstract

The majority of pharmaceutical medicines are developed as salt forms, and it is important to separate and detect the counterions as well as the parent pharmaceutical substances. It has been common practice to detect the organic pharmaceutical substances by reversed phase chromatography (RPLC) and separately the inorganic counterions by ion-exchange chromatography (IC). This method requires at least two analyses per sample. In this study, we demonstrate simultaneous detection of basic drug substances and inorganic anions in a single chromatographic analysis using a unique positively charged hydrophilic interaction chromatography (HILIC) stationary phase connected in series to a RPLC stationary phase. This combination of HILIC and RPLC phases shows different separations than the mixed-mode stationary phases, which has RPLC and anion-exchange functions.

Introduction

Pharmaceutical compounds often consist of hydrophobic drug component and counterion, making it challenging to analyze by HPLC in one single run. Although mixed-mode HPLC columns have been developed recently to separate acid, basic and neutral molecules in a single run, more affordable and robust approach will be needed. This study demonstrates simultaneous detection of basic drug substances and inorganic anions by serial connection of a unique HILIC phase to RP stationary phases.

Experimental

Stationary phases used

Column	COSMOSIL 5C18-MS-II	COSMOSIL HILIC
Stationary Phase	Octadecyl	Triazole
Average Particle Size	5 µm	5 µm
Average Pore Size	120 Å	120 Å
Surface Area	300 m²/g	300 m²/g

Triazole

Figure 1: Separation of organic compounds by reversed phase (RP) mode

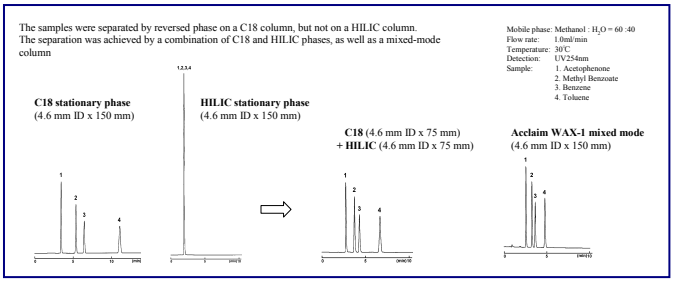


Figure 2: Separation of ions using ion-exchange chromatography (IC) mode

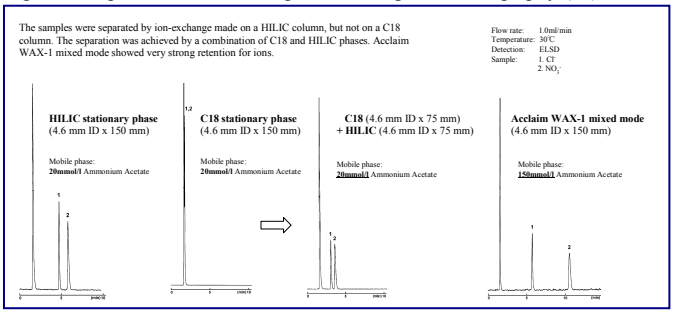


Figure 3: Effect of mobile phase

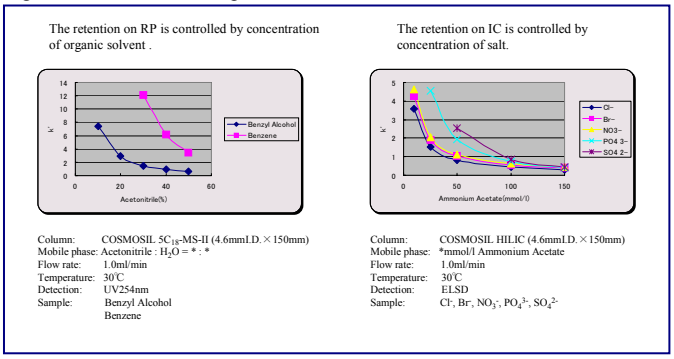


Figure 4: Simultaneous detection of basic drug substances and inorganic anions using a combination of HILIC and C18 columns

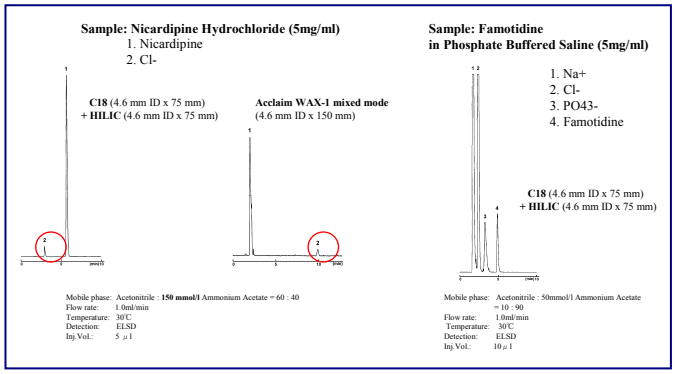


Figure 5: Changing the combination of columns

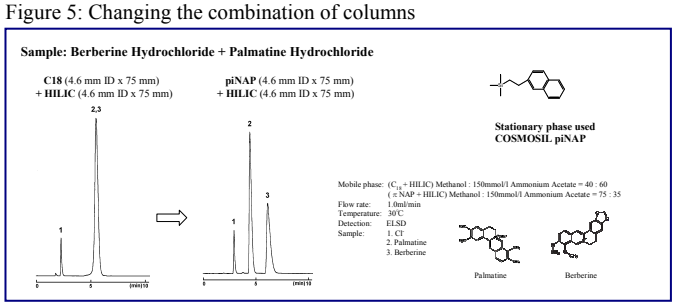
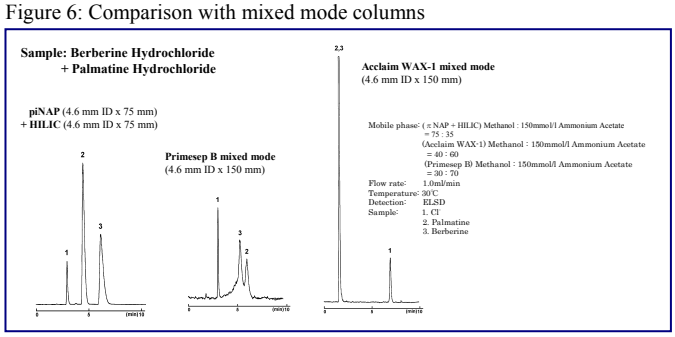


Figure 6: Comparison with mixed mode columns



Conclusions

- Selectivity based on hydrophobic and ionic properties can be achieved at the same time using a combination of RP columns and a positively charged HILIC stationary phase.
- Simultaneous separation of pharmaceutical substances and anionic counterions can be achieved with this method.
- The retention time can be controlled by concentration of organic solvent and buffer ionic strength.
- The combination of stationary phases and column length can be changed to obtain the optimum separation.