

SIMULTANEOUS SEPARATION OF PHARMACEUTICAL SUBSTANCES AND COUNTERIONS USING A SERIAL CONNECTION OF HYDROPHILIC INTERACTION CHROMATOGRAPHY 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 SC18-MS-II	COSMOSIL HILIC
Stationary Phase	Octadecyl	Triazole
Average Particle Size	5 μ m	5 μ m
Average Pore Size	120 Å	120 Å
Surface Area	300 m ² /g	300 m ² /g



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

The samples were separated by reversed phase on a C18 column, but not on a HILIC column. The separation was achieved by a combination of C18 and HILIC phases, as well as a mixed-mode column

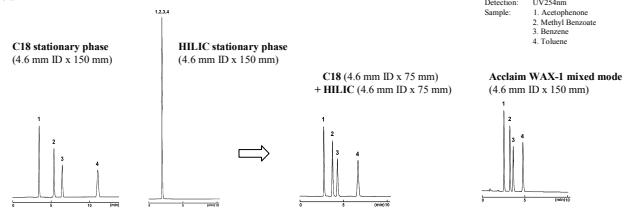


Figure 2: Separation of ions using ion-exchange chromatography (IC) mode

The samples were separated by ion-exchange made on a HILIC column, but not on a C18 column. The separation was achieved by a combination of C18 and HILIC phases. Acclaim WAX-1 mixed mode showed very strong retention for ions.

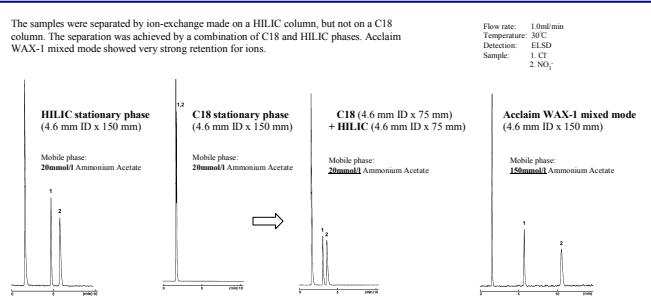
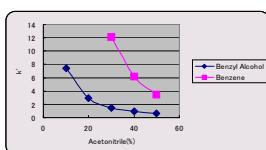
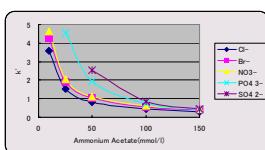


Figure 3: Effect of mobile phase

The retention on RP is controlled by concentration of organic solvent.



The retention on IC is controlled by concentration of salt.



Column: COSMOSIL 5C₁₈-MS-II (4.6mmID × 150mm)
Mobile phase: Acetonitrile : H₂O = * : *
Flow rate: 1.0ml/min
Temperature: 30°C
Detection: UV254nm
Sample: Benzyl Alcohol
Benzene

Column: COSMOSIL HILIC (4.6mmID × 150mm)
Mobile phase: *mMol/l Ammonium Acetate
Flow rate: 1.0ml/min
Temperature: 30°C
Detection: ELSD
Sample: Cl⁻, Br⁻, NO₃⁻, PO₄³⁻, SO₄²⁻

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

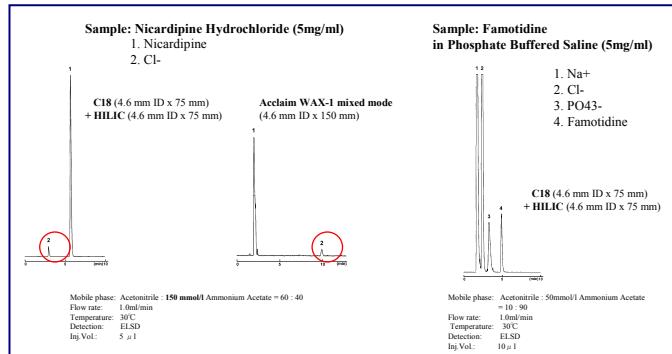


Figure 5: Changing the combination of columns

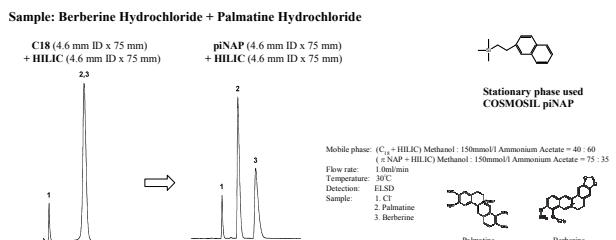
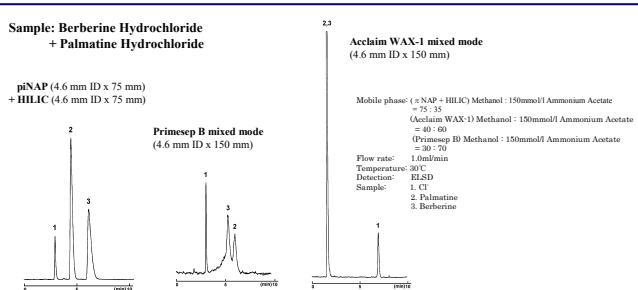


Figure 6: Comparison with mixed mode columns



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.