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Simultaneous Analysis of Active Pharmaceutical Ingredients and Their Counter-Ions Using a Mixed-Mode Column

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Introduction

Approximately 50 % of all drug molecules used in pharmaceutical products are reported to be ionic compounds. Ion chromatography is generally appropriate to analyze inorganic or organic ions, but not suitable for active pharmaceutical ingredients (APIs) analysis due to their hydrophobicity. On the other hand, reversed phase liquid chromatography (RPLC) is mainly applied for analysis of APIs, but cannot retain commonly-used ions for drugs. Consequently, it is difficult to analyze APIs and their counter-ions simultaneously.
In this study, we evaluated the ReDual column, our newly-developed mixed-mode column, for simultaneous analysis of APIs and their counter-ions. Mixed-mode column usage has increased because of the ability to analyze a wide range of compounds in a single run by multimode retention mechanisms. First, we investigated how retention behavior changes with parameters such as concentration of organic solvents, ion strength, and pH of the mobile phase using the ReDual CX-C18 mixed-mode column. Secondly, we analyzed naproxen sodium and potassium clavulanate. They were successfully analyzed on the column just by changing mixing ratio of the mobile phase.

Materials and Method

Reagents and standards

Reagents: Diclofenac sodium, naproxen sodium, potassium clavulanate, formic acid, and ammonium formate were purchased from Sigma-Aldrich. Water was made in house using a Millipore Milli-Q Advantage A10 Ultrapure Water Purification System. Acetonitrile was purchased from Honeywell.

Samples: Diclofenac sodium, naproxen sodium, and potassium clavulanate were separately dissolved in water to 1000 mg/L.

Methods

Samples were injected to a Shimadzu Nexera X2 UHPLC system consisting of two LC-30AD pumps (one of the pumps was equipped with low pressure gradient unit), DGU-20A5R degassing unit, SIL-30AC autosampler, CTO-30A column oven, ELSD-LT II evaporative light scattering detector, and CBM-20A system controller.

Active pharmaceutical ingredients and their counter-ions were separated on the ReDual CX-C18 mixed-mode column and detected by the ELSD-LT II. Fig. 1 and Table1 shows flow diagram and analytical conditions, respectively. The Nexera X2 allows a solvent blending function that can mix solvents according to desired ratios.

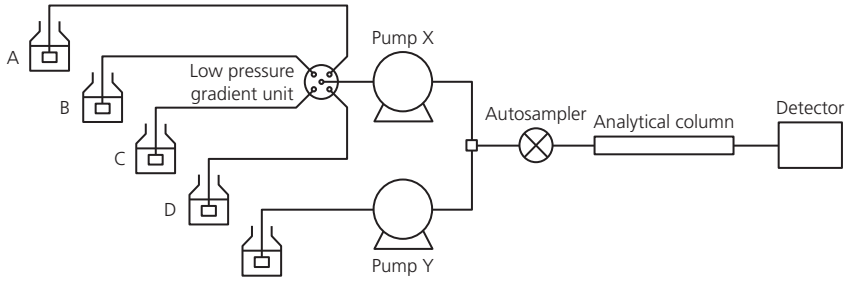

Fig.1 Flow diagram

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Table 1 Analytical conditions

System	: Shimadzu Nexera X2 UHPLC System
Column	: ReDual CX-C18 (150 mm L. x 4.6 mm I.D., 3 µm)
Mobile Phase X	: A: Water B: 100 mmol/L Formic acid in water C: 100 mmol/L Ammonium formate in water (Solvent blending function was used to prepare mobile phase X)
Mobile Phase Y	: Acetonitrile
Flow Rate	: 0.8 mL/min
Column Temperature	: 40 °C
Injection Volume	: 5 µL
Detection	: ELSD-LT II (Temp.: 40 °C, Gain: 6, Nebulizer gas: N_2, Gas pressure: 350kPa)

Results

Retention behavior

First, we investigated how retention behavior changes with parameters such as concentration of organic solvents, ion strength, and pH of the mobile phase using a 1000 mg/L diclofenac sodium solution (diclofenac: 928 mg/L, sodium: 72 mg/L) as a sample. The ReDual CX-C18 mixed-mode column has octadecyl and weak cation exchange groups that can retain hydrophobic compounds and cations simultaneously. When analyzing diclofenac sodium, diclofenac and sodium ion are retained by hydrophobic and ionic interaction, respectively as shown in Fig. 2. Therefore, their retentions can be controlled by concentration of organic solvents, ion strength, and pH of the mobile phase. Fig. 3 shows how each parameter affected retention behavior.

With the decrease of organic solvent:
Hydrophobic interaction becomes strong (factor of retention increase of diclofenac).
Concentration of ammonium ion in the mobile phase increases (factor of retention decrease of sodium ion).

With the increase of ion strength:
Concentration of ammonium ion in the mobile phase increases (factor of retention decrease of sodium ion).

With the decrease of pH (concentration of ammonium formate is kept constant):
Diclofenac becomes undissociated and hydrophobic (factor of retention increase of diclofenac).
The weak cation exchange group becomes undissociated (factor of retention decrease of sodium ion).

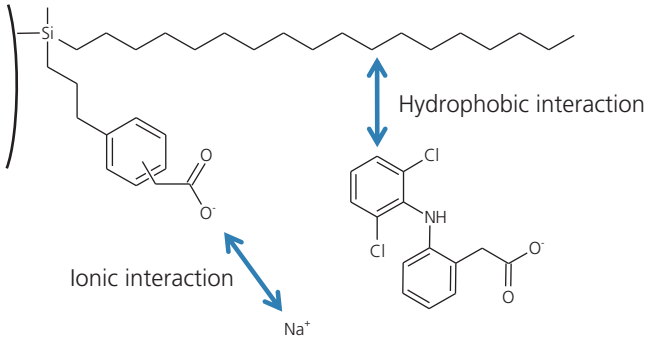

Fig.2 Retention mechanism of diclofenac and sodium ion on ReDual CX-C18

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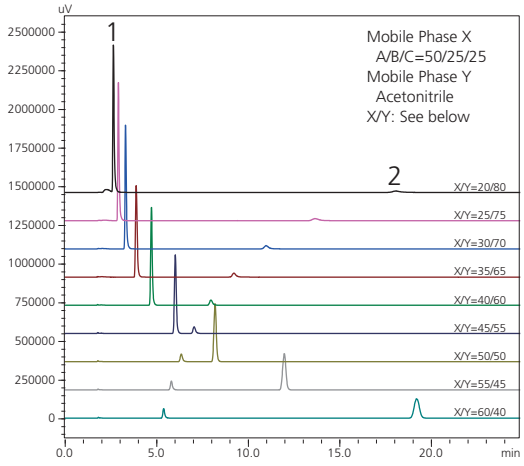
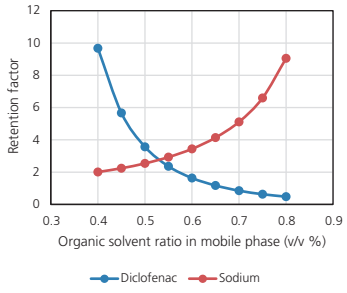
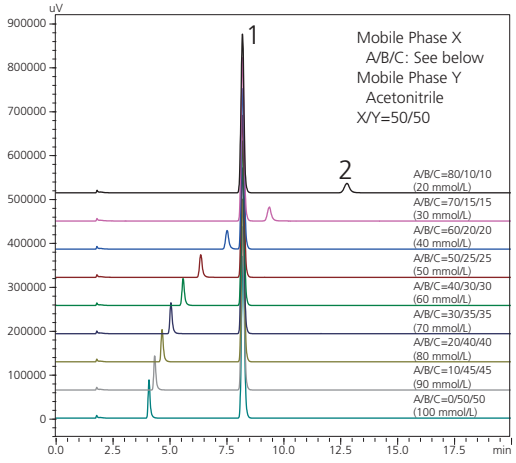
1. Changing concentration of organic solvent



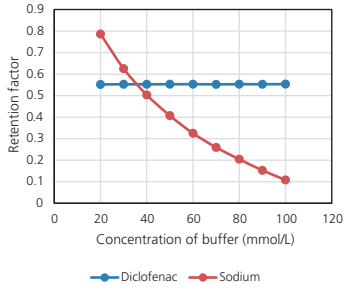
■ Peaks
1. Diclofenac
2. Na



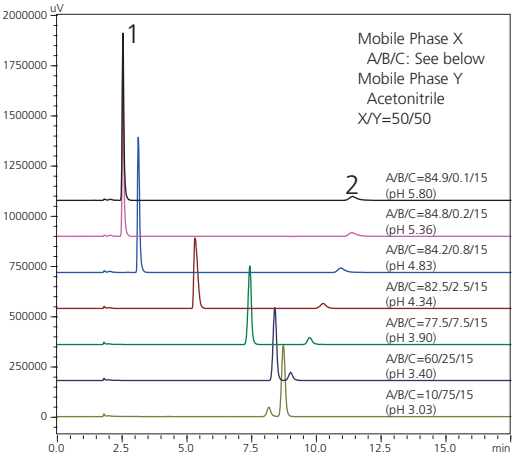
2. Changing ion strength



■ Peaks
1. Diclofenac
2. Na



3. Changing pH



■ Peaks
1. Diclofenac
2. Na

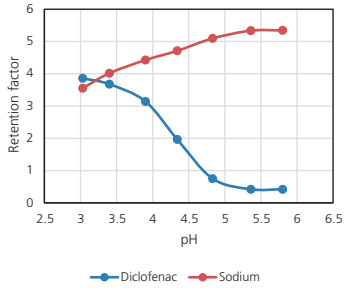


Fig.3 Retention behavior by changing parameters

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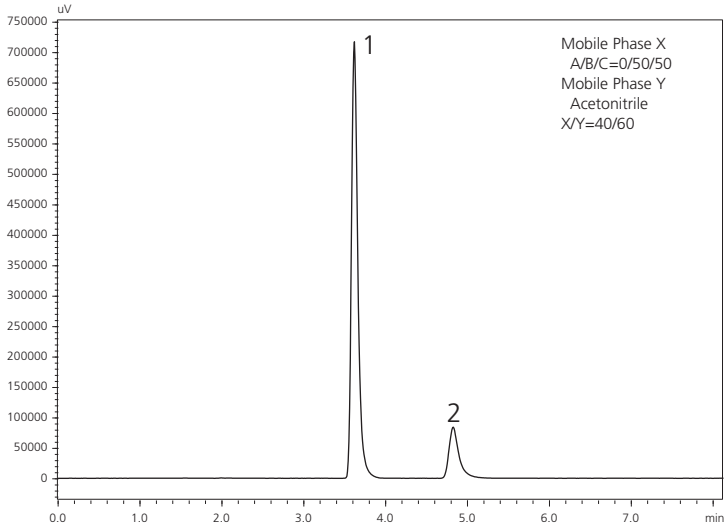
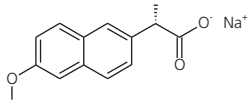
Application

This method was applied to analyses of naproxen sodium and potassium clavulanate. They were successfully analyzed just by changing mixing ratio of the mobile phases as shown in Fig. 4.

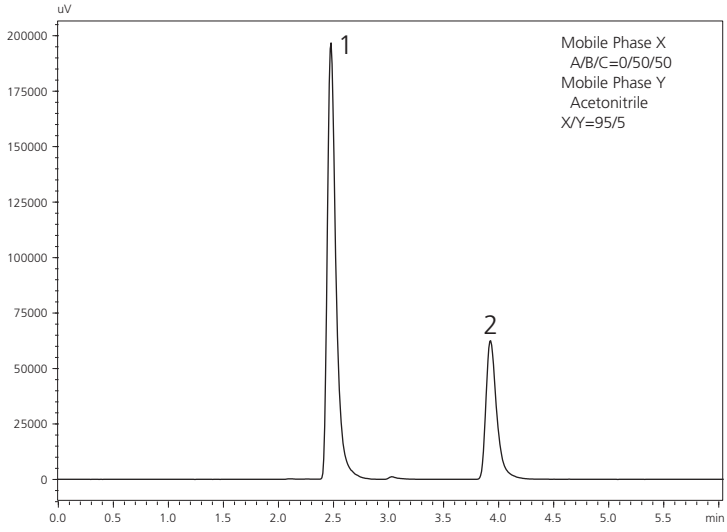
1. Analysis of naproxen sodium



■ Peaks
1. Naproxen
2. Na



2. Analysis of potassium clavulanate



■ Peaks
1. Clavulanic acid
2. K

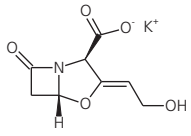


Fig.4 Chromatograms of naproxen sodium and potassium clavulanate

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Conclusions

- Retention behavior of an API and counter-ion on ReDual CX-C18 was investigated by changing concentration of organic solvents, ion strength, and pH of the mobile phase.
- Naproxen sodium and potassium clavulanate were successfully analyzed just by changing mixing ratio of the mobile phase.
- The Nexera X2 equipped with the solvent blending function was useful as a platform for method development by a mixed-mode column.

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