



CHIRAL ART

Chiral method optimization of ionic compounds
utilizing immobilized type column in reversed
phase mode

~ Influence of pH on retention behavior ~

YMC CO., LTD.

(F140718AE)

- Immobilized type CHIRAL ART columns can be used with various solvents that are commonly used for HPLC analysis.
- They can be used in both normal phase mode and reversed phase mode as they are compatible with non-aqueous and aqueous solvents (*).
- Reversed phase mode will be effective in case where a sample is hydrophilic and has limited solubility in organic solvent (e. g. Hexane)
- In this technical data sheet, we will introduce influence of pH of mobile phase on retention behavior of ionic compounds. We also introduce several example of chiral separation on reversed phase mode.

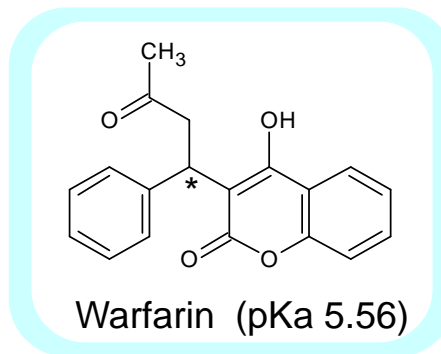
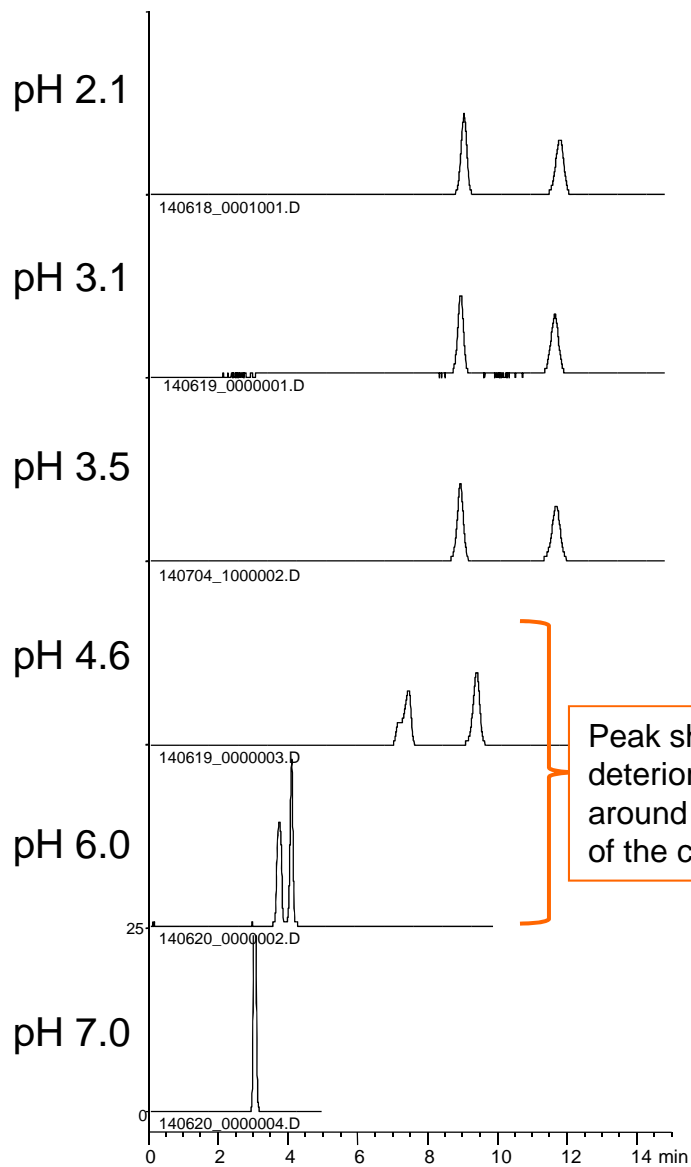
(*) We recommend that the column is dedicated to either phase. Frequent change of separation mode may result in short column lifetime.

Retention Behavior of Ionic Compound on Reversed Phase Mode

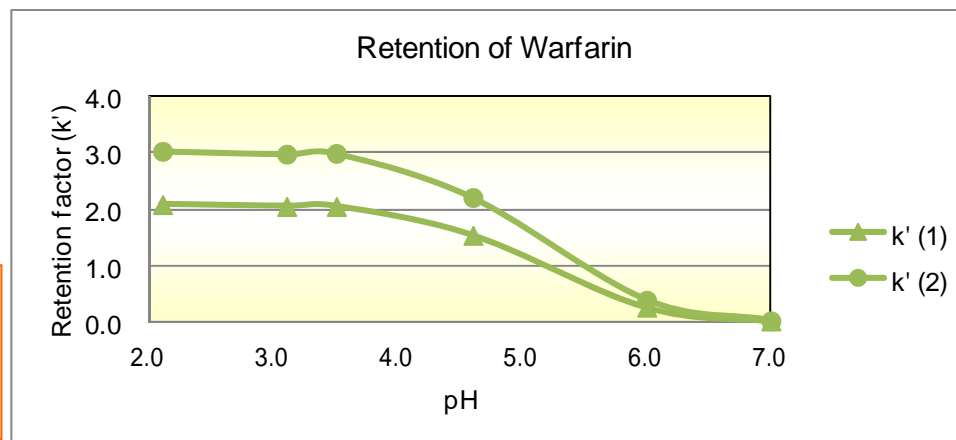
Acidic compound



Influence of pH on retention behavior



Column	: CHIRAL ART Cellulose-SB 5 μ m 250 X 4.6 mm.I.D.
Eluent	: 20 mM phosphate buffer/acetonitrile (50/50)
Flow rate	: 1.0 mL/min
Temperature	: 25
Detection	: UV at 254 nm
Injection	: 2 μ L (0.2 mg/mL)



Peak shape may deteriorated at around pKa value of the compound

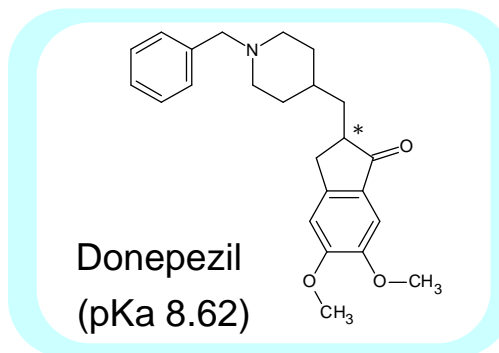
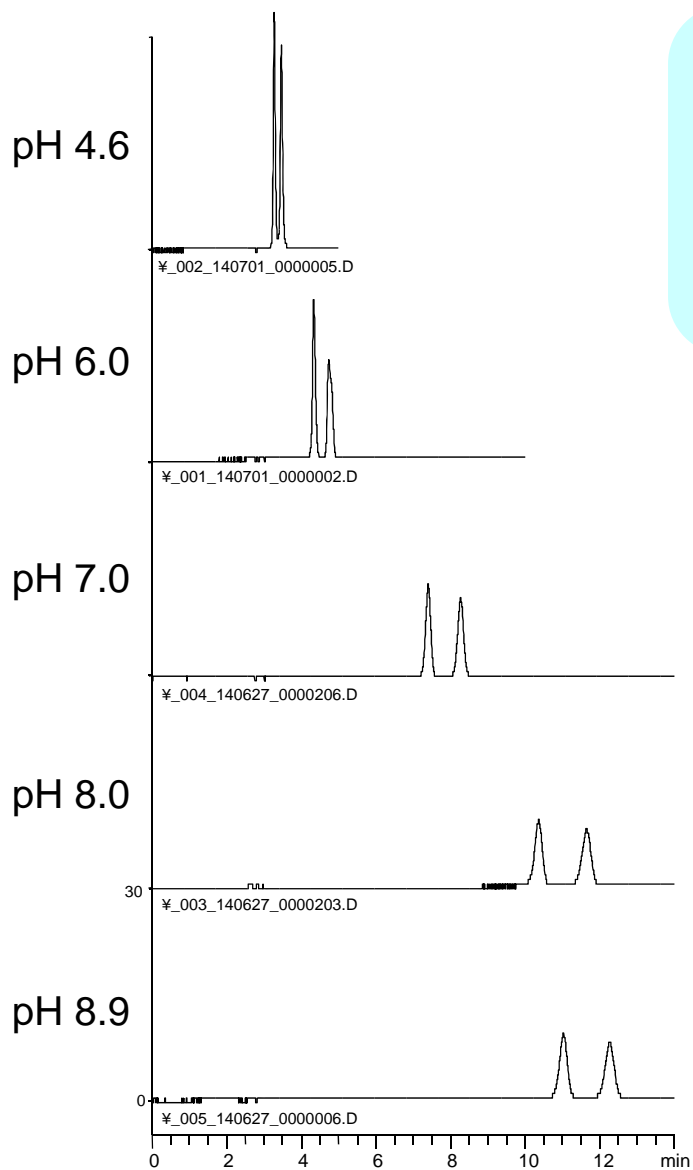
For acidic compound, retention is extended and good separation is achieved when decreasing the pH of a mobile phase (ionization was suppressed).

Retention Behavior of Ionic Compound on Reversed Phase Mode

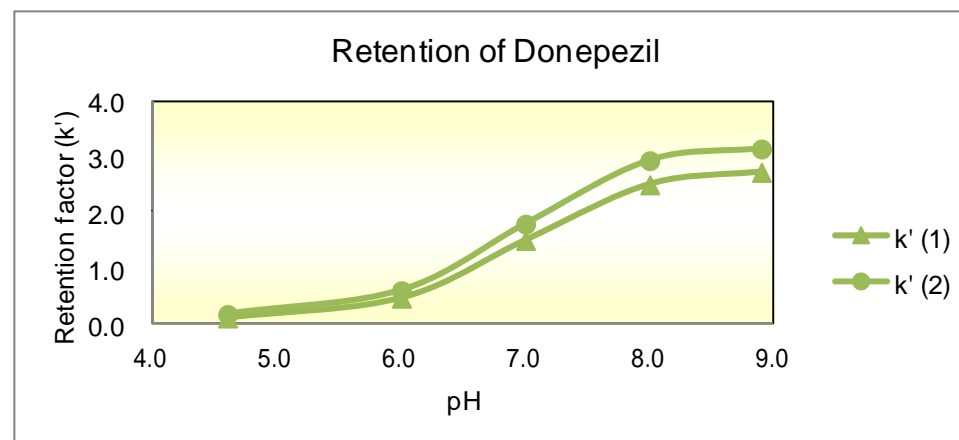
Basic compound



Influence of pH on retention behavior



Column	: CHIRAL ART Cellulose-SB 5 μ m 250 X 4.6 mm.I.D.
Eluent	: 20 mM phosphate buffer/acetonitrile (40/60)
Flow rate	: 1.0 mL/min
Temperature	: 25
Detection	: UV at 254 nm
Injection	: 2 μ L (0.2 mg/mL)



For basic compound, retention is extended and good separation is achieved when increasing the pH of a mobile phase (ionization was suppressed).

Tips for optimizing chiral separation method of ionic compounds on reversed phase mode



➤ Mobile phase

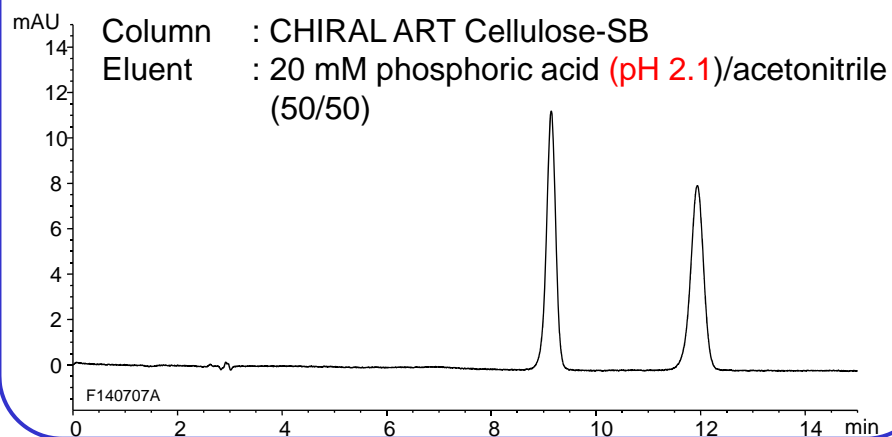
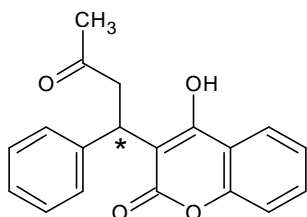
- > Optimal pH that ionization of analyte is suppressed is recommended.
(Retention will be extended and possibility of greater resolution is expected.)

➤ Column

- > Immobilized type CHIRAL ART columns are the best option.

Reversed phase analyses with optimized separation methods

Warfarin (pKa 5.56)



Donepezil (pKa 8.62)

