LC Technical Note
LT194
GL Sciences Inc.

Analysis of Phthalic Acid
Effect of Mobile Phase pH on Retention Time

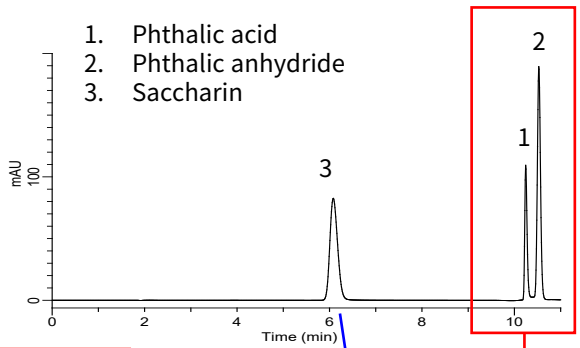
When the pH of mobile phase is changed in the analysis of reverse phase mode on HPLC, the dissociation / non-dissociation state changes depending on the compound, so the retention time changes. Normally, the retention time is longer in non-dissociated state and shorter in dissociated state. This time, using Inertsil WP300 C18, we analyzed the basic compound saccharin and the acidic compound phthalic anhydride standard, and found that the elution order changed under acidic and neutral conditions. We also report that it was estimated that phthalic anhydride in the sample was partially changed to phthalic acid. (R.Takahashi)

Measurement Examples of Saccharin and Phthalic Anhydride

1. Phthalic acid
2. Phthalic anhydride
3. Saccharin

Acidic conditions

: A) CH_3OH
B) 50 mM KH_2PO_4 (0.1% H_3PO_4 in H_2O)
(pH 2.6)

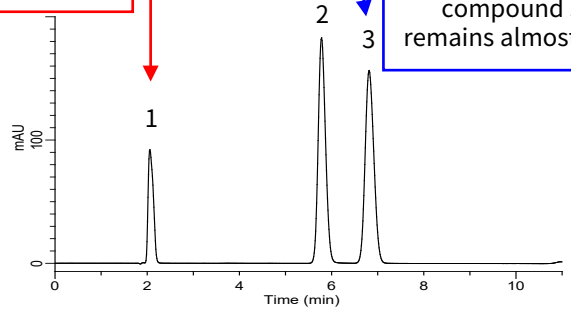


The retention of acidic compounds phthalic acid and phthalic anhydride varies greatly depending on the acidic and neutral conditions.

The retention of the basic compound saccharin remains almost unchanged.

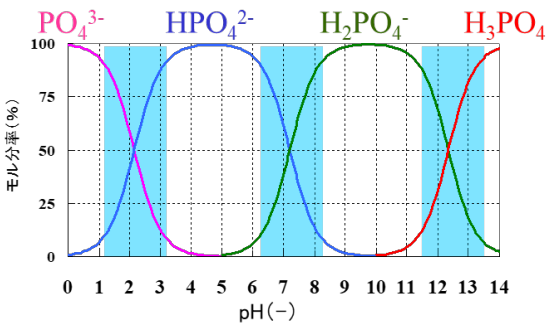
Neutral condition

: A) CH_3OH
B) 50 mM K_2HPO_4 (0.1% H_3PO_4 in H_2O)
(pH 6.7)

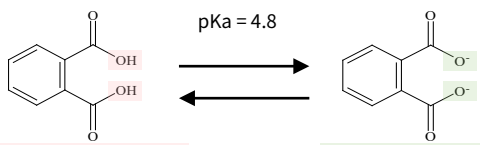


For the analysis conditions, we referred to the 18th revised Japanese Pharmacopoeia draft (acidic conditions) and the 18th revised Japanese Pharmacopoeia (neutral conditions).

Phosphoric Acid Buffering Action and Phthalic Acid Dissociation



Phthalic Acid



Acidic conditions
Dissociation subsides and retention becomes stronger.

Neutral condition
Retention is weakened due to dissociation.

Phosphoric acid has a higher buffering capacity near pH = 2.15, 7.20, 12.35. Even with the same phosphate buffer solution, the retention of acidic compounds is strong under acidic conditions, while the retention of basic compounds is strong under basic conditions.

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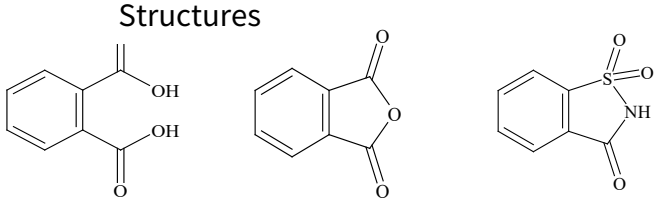
HPLC condition

Column	: Inertsil WP300 C18 5 µm, 150×4.6 mm I.D.
Flow rate	: 1.0 mL/min
Column Temp.	: 20 °C
Detection	: UV 230 nm
Inj. Vol.	: 10 µL
Gradient Cond.	: The solvent is described on the previous page.

Time(min)	A(vol%)	B(vol%)
0	10	90
7.0	10	90
8.0	95	5
11.0	95	5

Chemical Structures

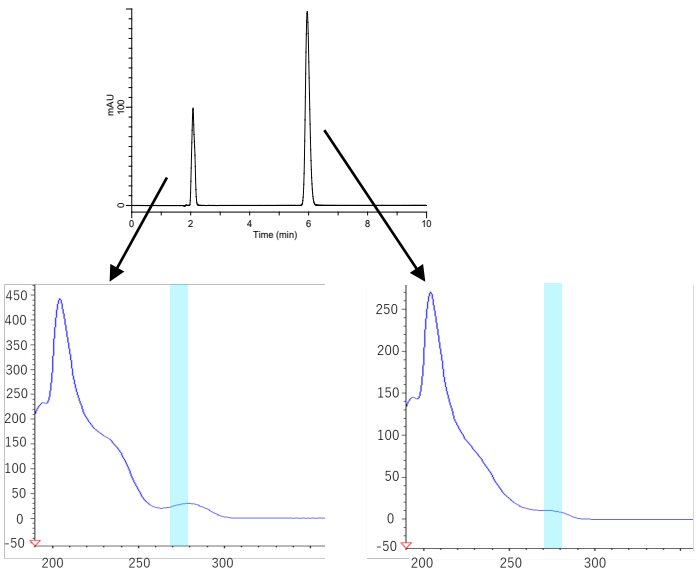


Phthalic acid　Phthalic anhydride　Saccharin

Structures are created using Chemistry 4-D Draw which is provided by ChemInnovation Software, Inc.

Phthalic anhydride standard product

When the standard phthalic anhydride was measured, two peaks were seen, and it was confirmed that they were phthalic anhydride and phthalic anhydride, respectively.



Phthalic acid　Phthalic anhydride

Comparing phthalic acid and phthalic anhydride, there was a difference in the absorption curves around 270 nm to 280 nm.

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