Unlocking the Potential of Basic Compounds Analysis Through pH Optimization Using a High Alkali-Resistant Column in RPLC Analysis

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Introduction

Silica-based columns have been the most widely used in reversed-phase liquid chromatography (RPLC) due to their advantages, such as high separation efficiency and mechanical strength. However, silica is easily eroded when exposed to alkaline solutions. Therefore, when using silica-based columns, the pH range of the eluent is limited.
L-column3 is an all-around column for RPLC, that has significantly improved the chemical stability of the packing, due to the newly developed, highly alkali-resistant PCS (perfectly chemically stable) silica and the further advanced end-capping.
This enhancement enables the use of an alkaline eluent with *L-column3*. This poster introduces improvements in the analysis method for basic compounds using *L-column3* and alkaline eluent in RPLC analysis.

Experimental

We conducted the following tests to evaluate the performance of *L-column3* and improved the analysis method for basic compounds using *L-column3*.

- Durability test at pH 1 and pH 12
- Comparison of column performance between *L-column3* and competitor column

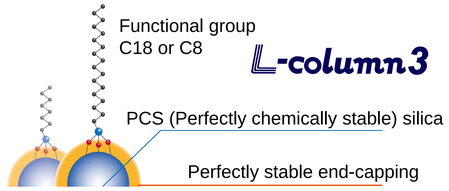
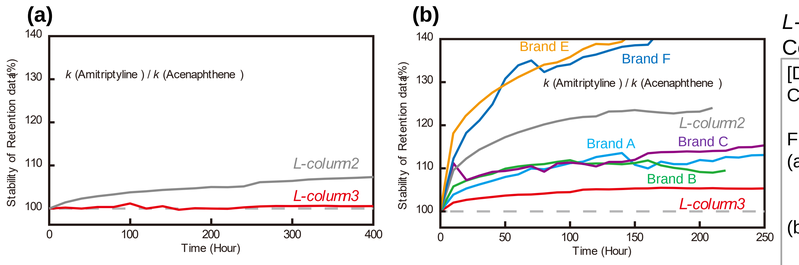


Fig. 1 Image of *L-column3* packing

Result and Discussion

Tests to evaluate the performance of *L-column3*

Durability test at pH 1 and pH 12



L-column2 is usable pH from 1 to 9.
Competitor columns are usable up to pH 12.

[Durability test condition]
Column: *L-column3 C18*, 5 µm,
2.1 mm I.D., 150 mm L.
Flow: 0.2 mL/min
(a) Eluent: Methanol/1% Trifluoroacetic acid in water (10/90)
Column Temp. : 40℃
(b) Eluent: Methanol/54 mmol/L Triethylamine in water (10/90)
Column Temp. : 50℃

Fig. 2 Durability test at (a)pH 1 and (b)pH 12

L-column3 exhibited exceptionally high alkali-resistance and maintained its performance even after prolonged exposure to a pH 12 and 50℃. It also showed high stability under acidic condition.

Comparison of column performance between *L-column3* and competitor columns

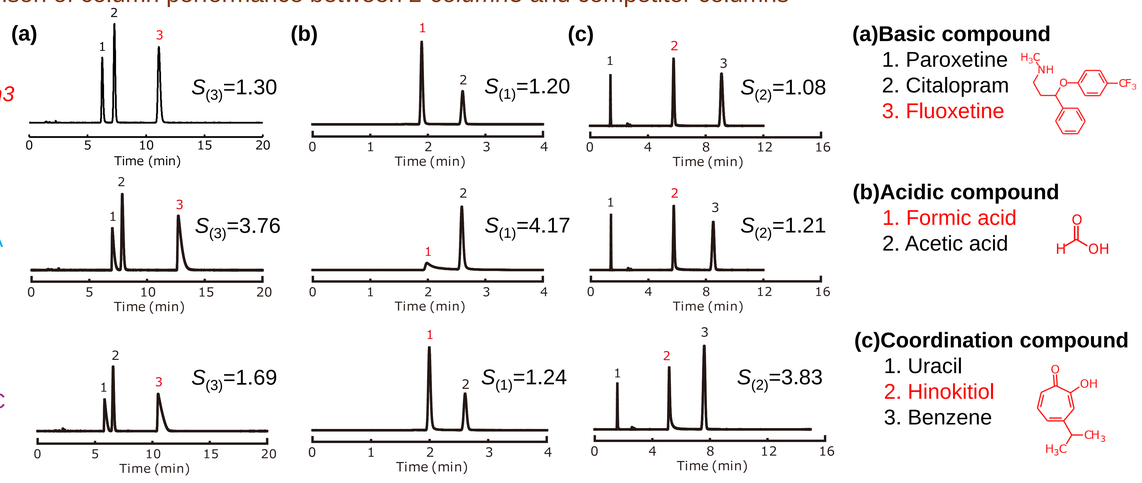
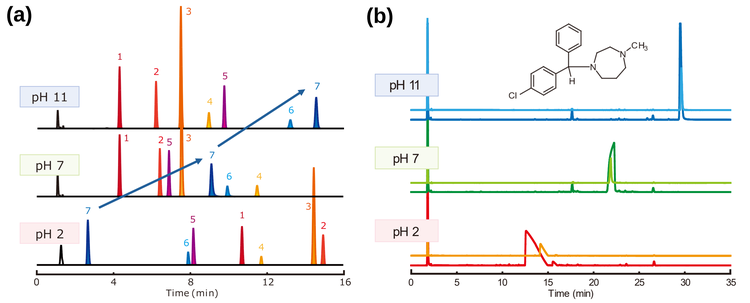


Fig. 3 Comparison of column performance between *L-column3* and competitor columns
Eluent: (a) Acetonitrile/25 mmol/L Phosphate buffer pH7 (35/65, v/v), (b) Acetonitrile/20 mmol/L Phosphoric acid in water (2/98, v/v), (c) Acetonitrile/20 mmol/L Phosphoric acid in water (60/40, v/v)

Due to its superior low adsorption characteristics, *L-column3* produced sharp peaks not only for basic compounds but also for acidic and coordination compounds.

Improvements of RPLC analysis method for basic compound using *L-column3*

Change in retention behavior and improvement in peak shape of basic compounds due to pH



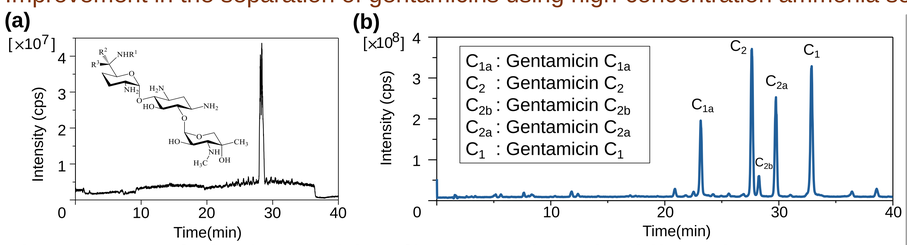
【(a)Analytical conditions】
Column: *L-column3 C18*, 5 µm, 2.1 mm I.D., 150 mm L.
Eluent: A; Acetonitrile, B; 25 mmol/L Phosphate buffer (pH 2, pH 7, pH 11)
A/B; 20/80-70/30（0-20 min）
Flow: 0.3 mL/min
Analyte:1. ketoprofen (Acidic compound), 2. Ibuprofen (Acidic compound), 3. Indomethacin (Acidic compound), 4. Isobutyl 4-hydroxybenzoate (Weak acidity compound), 5. Fexofenadine (Amphoteric compound), 6. Fluvoxamine (Basic compound), 7. Triprolidine (Basic compound)

Fig. 4 Effect of eluent pH on (a)selectivity for active pharmaceutical ingredients and (b)peak shape when overload injection for Homochlorcyclizine

【(b)Analytical conditions】
Column: *L-column3 C18*, 5 µm, 2.1 mm I.D., 150 mm L.
Injection volume: upper; 0.5 µL, lower; 5 µL

Changing the pH of the eluent altered the retention behavior of dissociable substances. Using an alkaline eluent significantly improved the peak shapes of basic compounds.

Improvement in the separation of gentamicins using high-concentration ammonia solution

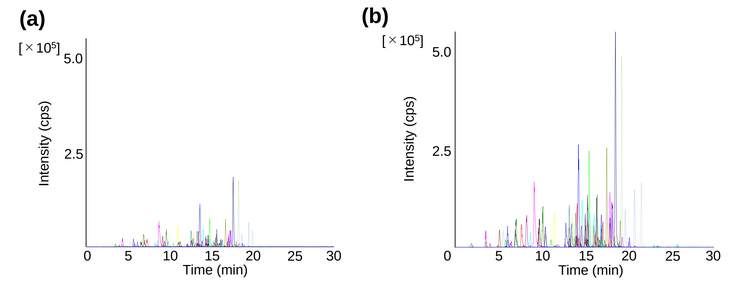


【(b)Analytical conditions】
Column: *L-column3 C18*, 3 µm, 2.0 mm I.D., 150 mm L. Metal-free column
Eluent: A; Acetonitrile, B; Water/28% Ammonia solution/Acetic acid（200/20/0.5, v/v/v, pH 11.2), A/B; 1/99-15/85 (0-40 min)
Flow rate: 0.2 mL/min
Sample: 100 µg/mL Gentamicin sulfate
Column Temp. : 40℃
Injection volume: 10 µL

Fig. 5 LC-MS analysis of gentamicin sulfate separation using (a)ion-pair reagent (heptafluorobutyric acid) or (b)ammonia solution

When analyzing gentamicin sulfate using ammonia solution, it was detected 5 gentamicins and 22 impurities presumed to be gentamicin-related compounds. Gentamicin's pK_a is 9.8, which suggests that it was in non-ionized form in the alkaline eluent. It is conceivable that the ammonium ions interacted with silanol groups, inhibiting the adsorption of gentamicins to these sites.

Improvement in the sensitivity of 120 residual pesticides in LC-MS/MS analysis using alkaline solution



【Analytical conditions】
Column: *L-column3 C18*, 3 µm, 2.1 mm I.D., 150 mm L.
Analyte concentration: 50 ppb
Injection volume: 5 µL
(a)Eluent: A; 5 mmol/L Ammonium acetate solution (pH 7.0)
B; Methanol (containing 5 mmol/L Ammonium acetate)
(b)Eluent: A; 5 mmol/L Ammonia solution (pH 10.5)
B; Methanol (containing 5 mmol/L Ammonia)
Detection: ESI-MS/MS (+)

Fig. 6 LC-MS/MS analysis of residual pesticides using (a) an ammonium acetate solution or (b) an ammonia solution

Using an ammonia solution improved the sensitivity for 119 out of 120 pesticides.
Compared to using an ammonium acetate solution, the sensitivity with ammonia solution was on average 3.9 times (*S/N*) higher.
It is conceivable that the ionization efficiency of the eluent is affected the sensitivity of residual pesticides.

Conclusion

L-column3 is an all-around column for RPLC, having its stability and low adsorption. It allows for the choice of eluent pH from 1 to 12.
Using an alkaline eluent in RPLC analysis of basic compounds offers many benefits in terms of separation, peak shape, loading and sensitivity.
L-column3 significantly contributes to the use of alkaline eluent.