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Application News

No.L447

High Performance Liquid Chromatography

Analysis of Compounds Containing a Phosphate Group Using the New Mastro™ High Pressure-Resistance Stainless Steel-Free LC Column

Substances containing phosphate groups or metal chelates are often found in pharmaceuticals and metabolites. When subjected to HPLC analysis, these compounds are known to adsorb to metallic surfaces in the HPLC system flow line, thereby causing adverse peak tailing in their chromatograms. The typical remedy for this has been to add chelating agents and salts to the mobile phase, but the use of such nonvolatile eluents is not applicable when using an LC/MS as the detector.

Here, we examined the effects of different flow line materials on the peak shape of coordination compounds. Also, using a formic acid mobile phase, which is commonly used in LC/MS analysis, we examined the inhibiting effect on metal adsorption of coordination compounds that is provided with Mastro™ high pressure-resistance stainless steel-free analytical column.

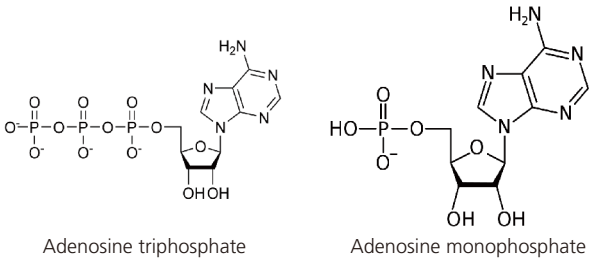

Fig. 1 Structural Formulas of Nucleotides

Table 1 Analytical Conditions

Column	: Typical C18 (100 mm L. × 2.1 mm I.D., 3 µm)
Instrument	: Nexera
Mobile Phase	: 10 mmol/L Ammonium Formate in Water:Acetonitrile=99:1
Flowrate	: 0.2 mL/min
Column Temp.	: 40 °C
Detection	: SPD-M20A at (254 nm)
Injection Vol.	: 1 µL

Influence of HPLC Tubing Material

When using mobile phases applicable to LC/MS analysis, adsorption to stainless steel tubing of any coordination compound that may be present can result in peak tailing. Thus, using a mobile phase applicable to LC/MS, together with a conventional column, we investigated the effect of the material used for the autosampler outlet tubing (600 mm) on peak shape in the measurement of a nucleotide standard solution. Stainless steel and PEEK materials were both used for the tubing.

A nucleotide is a compound consisting of a nucleoside that contains one or more phosphate groups. ATP (adenosine triphosphate) is a compound that contains three such phosphate groups (Fig. 1). We prepared solutions of ATP and AMP (approximately 10 mg/L each) and conducted analysis using the analytical conditions shown in Table 1.

When using stainless steel tubing, the delay in retention time due to adsorption was verified, but regardless of the tubing material used, no significant difference in tailing due to adsorption was observed. It is therefore presumed that the column plays a larger role in the occurrence of peak tailing.

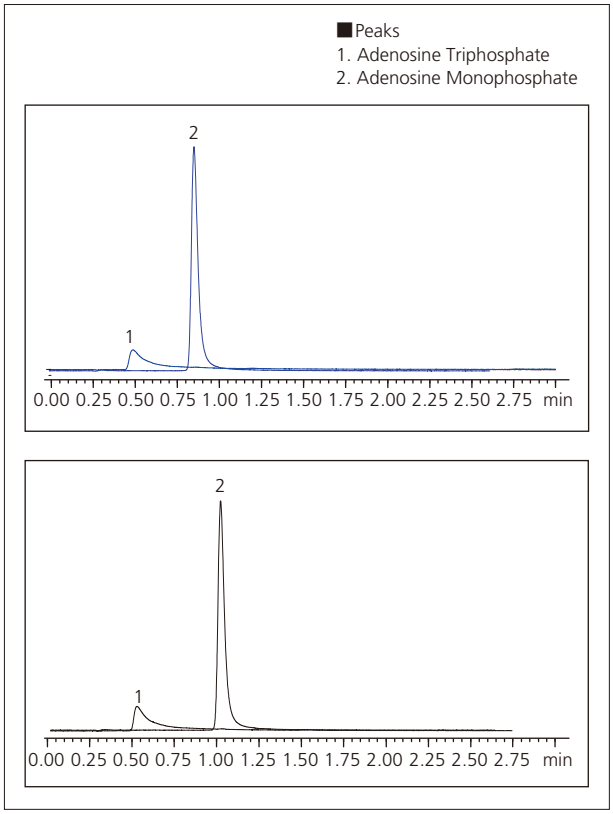

Fig. 2 Difference in Peak Shape Due to Tube Material (Upper: PEEK Material, Lower: Stainless Steel)

Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium Succinate Analysis

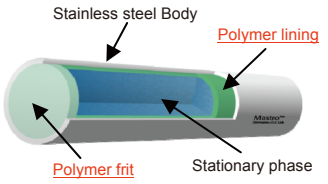

Fig. 3 Mastro™ Column Image

Next, we investigated the effect of the Mastro™ stainless steel-free column on retention time shift and peak tailing. The high pressure-resistance stainless steel-free Mastro™ column consists of a stainless steel body with a polymer-coated inner surface, and a frit which adopts a highly cross-linked polymer material. As metallic activity is reduced to the greatest extent possible, adsorption in the column of substances susceptible to metal coordination is greatly suppressed (Fig. 3).

The main component of an adrenocorticotropic hormone preparation is hydrocortisone sodium phosphate, which contains one phosphate group. On the other hand, the main component of the water-soluble hydrocortisone preparation is hydrocortisone sodium succinate, which does not contain a phosphate group (Fig. 4).

We conducted analysis of these compounds using a mobile phase that is applicable to LC/MS. Table 2 shows the analytical conditions.

With a typical C18 column, tailing is generally seen in analysis of hydrocortisone sodium phosphate, with delayed retention time and peak reversal due to adsorption. With the Mastro™ column, however, sharp peak shapes were obtained for both substances (Fig. 5).

Thus, we confirmed that the column plays a large role in the suppression of peak tailing of coordination compounds when using a mobile phase applicable to LC/MS, and that a stainless steel-free column is effective in this respect.

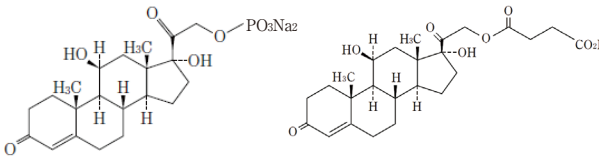

Fig. 4 Structure of Hydrocortisone

The data and examples of analysis of the adrenocorticotropic hormone and aqueous hydrocortisone preparations were obtained in collaboration with Associate Professor Kenichiro Todoroki, Laboratory of Analytical and Bio-Analytical Chemistry, School of Pharmaceutical Sciences, University of Shizuoka.

Table 2 Analytical Conditions

Column	: Mastro™ C18 (100 mm L. × 2.1 mm I.D., 3 µm) Typical C18 (100 mm L. × 2.1 mm I.D., 3 µm)
Instrument	: Nexera
Mobile Phase	: 0.1 % Formic Acid in Water /0.1 % Formic Acid in Acetonitrile = 50:50
Flowrate	: 0.2 mL/min
Column Temp.	: 40 °C
Detection	: SPD-M20A at (254 nm)
Injection Vol.	: 1 µL

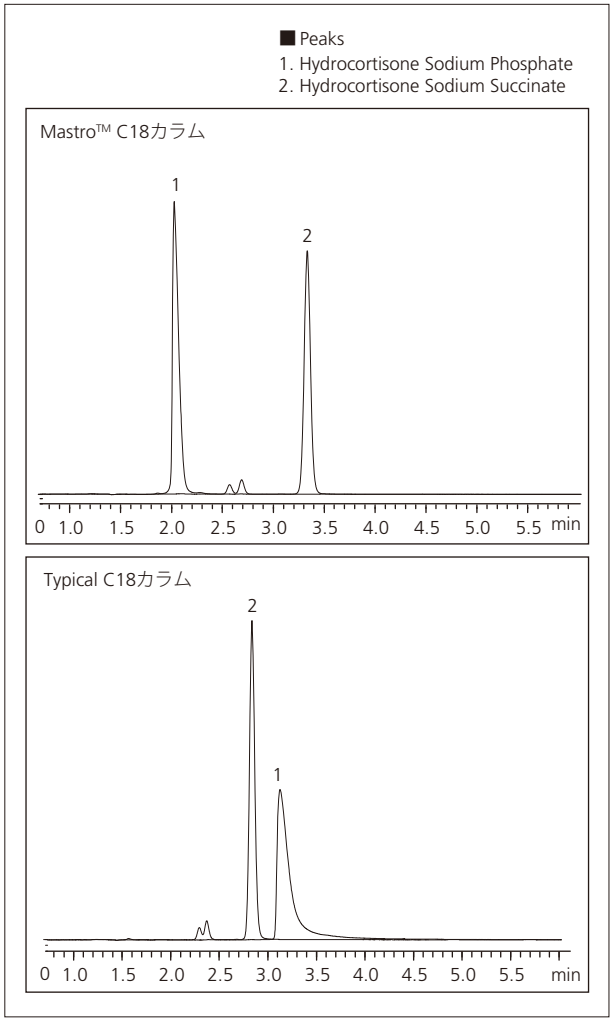

Fig. 5 Chromatograms of Hydrocortisone Sodium Phosphate and Hydrocortisone Sodium

For more information about the Mastro™ column, please contact Shimadzu GLC Ltd.

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