<u>Analysis of Coenzyme Q10 in a</u> <u>Supplement (C18M 4D)</u>

Coenzyme Q_{10} , also known as ubiquinone Q_{10} (or ubiquinone), is a fat-soluble substance found in foods such as meat and seafood. It is expected to have antioxidant and anti-aging effects and is also known as a vitamin-like substance. In this application, we analyzed a commercially available supplement (soft capsule) using a silica-based reversed-phase chromatography column, <u>Silica C18M 4D</u>. C18M 4D was feasible analyzing coenzyme Q_{10} without having interference from other components. In addition, obtained calibration curve prepared using standard samples showed a highly linear calibration curve with a coefficient of determination (R²) 0.9991, in the concentration range of 10 to 1000 mg/L.

Sample pretreatment

- 1. Remove the powder contents of the supplement (soft capsule).
- 2. Add 4 mL of eluent to 5 mg of the sample. Apply sonication for 10 minutes,
- and then heat at 40 \square for 2 minutes to dissolve the sample completely.
- 3. Let it cool to room temperature and make up to 5 mL with eluent.
- 4. Filter with 0.45 μm filter and use it as a sample.

Sample: 5 μL (Sample solution) (Standard solution) 1000 mg/L in eluent

1. Coenzyme Q_{10}



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Column[Shodex Silica C18M 4D (4.6 mm I.D. x 150 mm) Eluent[CH30H/C2H50H=40/60 Flow rate[1.0 mL/min Detector[UV (275 nm) Column temp.[]35 []

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Detector∐UV (275 nm)

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Angiotensins

Angiotensins were analyzed using Asahipak ODP-50 6D (a column for reversed phase chromatography).



Column : Shodex Asahipak ODP-50 6D (6.0mmID*150mm) Eluent : 0.05% TFA aq./CH₃CN=80/20 Flow rate : 1.0mL/min Detector : UV(220nm) Column temp. : 30°C

Standard Proteins (4) (C4P-50 4D)

Three kinds of protein standards were analyzed using Asahipak C4P-50 4D (a column for reversed phase chromatography).



- 2. Chymotrypsinogen A
- 3. Ferritin

Column : Shodex Asahipak C4P-50 4D (4.6mmID*150mm) Eluent : (A); 0.1% TFA aq./CH₃CN=90/10 (B); 0.1% TFA aq./CH₃CN=5/95 Linear gradient: 0 to 30min, (A) to (B) Flow rate : 0.6mL/min Detector : UV(280nm) Column temp. : 30°C

<u>Analysis of Lactoferrin Supplement</u> (C4P-50 4D)

Lactoferrin is a protein found in many mammalian milks, especially in breast milk. In this application, a supplement containing lactoferrin was analyzed using a polymer-base reversed phase column, <u>Asahipak C4P-50 4D</u>. C4P-50 4D was proved to effectively detect lactoferrin in a supplement. It also provided a good linear calibration curve in the concentration range 20 to 4,000 mg/L.

(Sample Preparation)

- 1) Grind three tablets of a commercial lactoferrin supplement in a mortar.
- 2) Measure out 0.2 g of the powder and add 50 mL of ultra-pure water.
- Sonicate for 10 minutes.
- 3) Centrifuge and filter it with a No.5B filtering paper.
- 4) Discard the first 10 mL and collect the rest.
- 5) Filter using a 0.45- μ m membrane filter and use it as the injection sample.





Sample : 25 μL 1. Lactoferrin (Standard solution) 1000 mg/L

Column : Shodex Asahipak C4P-50 4D (4.6 mm I.D. x 150 mm) Eluent : (A); $H_20/TFA=100/0.05(v/v)$ (B); CH₃CN/TFA=100/0.05(v/v) Linear gradient: 0 to 50 % B (0 to 10min), 50 % B (10 to 15 min), □□□□□ 50 to 0 % B (15 to 16 min), 0% B (16 to 20 min) Flow rate : 0.8 mL/min Detector : UV (280 nm) Column temp. : 40 °C

<u>Analysis of Lactoferrin Concentrate</u> <u>According to Analytical Methods of</u> <u>Food Additives in Foods (C4P-50 4D)</u>

Lactoferrin is a protein found in many mammalian milks, especially in breast milk. The Japanese Analytical Methods of Food Additives in Foods lists an analysis method for lactoferrin and specifies to use a butylated polyvinyl alcohol polymer gel column. In this application, a polymer-base reversed phase column, <u>Asahipak C4P-50 4D</u>, was used to analyze lactoferrin according to the Analytical Methods of Food Additives in Foods. A lactoferrin concentrate was analyzed as an actual sample. The Analytical Methods of Food Additives in Foods states that lactoferrin concentrates to contain 85% or more of lactoferrin. It was confirmed that the sample analyzed met this requirement.

(Sample Preparation)

- 1) Measure out 0.1 g of a commercial lactoferrin concentrate.
- 2) Add 50 mL of 0.3 % NaCl solution to dissolve the concentrate.
- 3) Use the dissolved-solution as the injection sample.





Column : Shodex Asahipak C4P-50 4D (4.6 mm I.D. x 150 mm) Eluent :(A); 0.3 % NaCl/CH₃CN/TFA=9000/1000/3 (B); 0.3 % NaCl/CH₃CN/TFA=5000/5000/3 Linear gradient: 50 to 100 % B (0 to 25 min) Flow rate : 0.8 mL/min Detector : UV (280 nm) Column temp. : 40 °C

<u>Analysis of Lactoferrin Supplement</u> (C4P-50 4D)

Lactoferrin is a protein found in many mammalian milks, especially in the first breast milks. In this application, a supplement containing lactoferrin was analyzed using a polymer-base reversed phase column, <u>Asahipak C4P-50 4D</u>. C4P-50 4D was proved to effectively detect lactoferrin in a supplement. It also provided a good linear calibration curve in the concentration range 20 to 4,000 mg/L.

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