RESEARCH REPORT FEBRUARY 2017

1. MATERIALS AND METHODS

SE HPLC (Size Exclusion HPLC)

In SEC-HPLC, compounds are separated based on the size of the molecule, i.e. the hydrodynamic volume (radius of gyration), where molecules with higher radii elute before molecules with smaller radii.

Conditions:

- Column: TSKGel SuperSW3000 4.6x300mm, 4 µm (Tosoh, 18675)
- Column Temp: 25 °C
- Mobile Phase: 0.2 M Phosphate Buffer, pH 6.8
- Flow Rate: 0.35 mL/min
- Auto Sampler Temperature: 4°C
- Detection: UV at 280 nm, 16 nm bandwith, reference off
- Sample preparation: dilute to 10 mg/mL with formulation buffer

Preparation of reagents:

Mobile Phase Buffer preparation - dissolve 12.2 g/L sodium phosphate monobasic dihydrate and 32.7 g/L sodium phosphate dibasic heptahydrate in 950 mL/L purified water. Mix well using a stir bar. Adjust pH to a target of 6.8 ± 0.2 with sodium hydroxide or phosphoric acid. Adjust to volume to 1L with purified water. Filter through a 0.22µm filter. Storage: Room temperature.

HIC HPLC (Hydrophobic Interaction Chromatography HPLC)

Hydrophobic Interaction Chromatography (HIC) is a method of separating proteins by the degree of nonpolar (hydrophobic) regions. Proteins are loaded onto the column under high salt conditions and a "reverse salt gradient" is used to elute the sample. The most hydrophilic species are eluted first followed by species of higher hydrophobicity. The elution time of proteins is directly proportional to their degree of hydrophobicity.

Chromatographic parameters

Column:	TSKgel Butyl-NPR column, Tosoh Bioscience
In-line filter:	0.5µm
Equilibration of column:	10% mobile phase A and 90% Mobile Phase B
Elution mode:	Gradient according to Table 1
Flow:	0.8 mL/minute

Sample preparation: Dilute to 4 mg/mL using Formulation buffer
Sample temperature: 4°C± 2°C
Column temperature: 40°C± 2°C
Detection: 280 nm and 360nm

Table 1 Gradient table 1

Time [minutes]	**Flow** [mL/minute]	**Eluent A** [%]	**Eluent B** [%]
0.0	0.8	10	90
1.0	0.8	10	90
10.0	0.8	100	0
13.0	0.8	100	0
14.0	0.8	10	90
20.0	0.8	10	90

Preparation of Reagents

5 M phosphoric acid (H_3PO_4)

Add 66mL purified water to a 100 mL measuring cylinder or volumetric flask
Add 34mL phosphoric acid carefully.
Mix well
Store the solution at room temperature for up to 6 month.

Mobile Phase A, 50 mM Sodium Phosphate (pH 6.0±0.2), 1000mL

Add 7.10 g sodium phosphate dibasic, to a 1000mL beaker
Add approximately 800mL Milli-Q water
Mix well and adjust pH to 6.0±0.2 with 5M phosphoric acid
Transfer the solution to a volumetric flask and fill to volume with Milli-Q water
Filter the solution through 0.2 µm filter.
Store the mobile phase buffer at room temperature for up to 1 month.

Mobile Phase B, 50mM Sodium Phosphate, 850 mM Sodium Citrate (pH 6.0±0.2), 1000mL

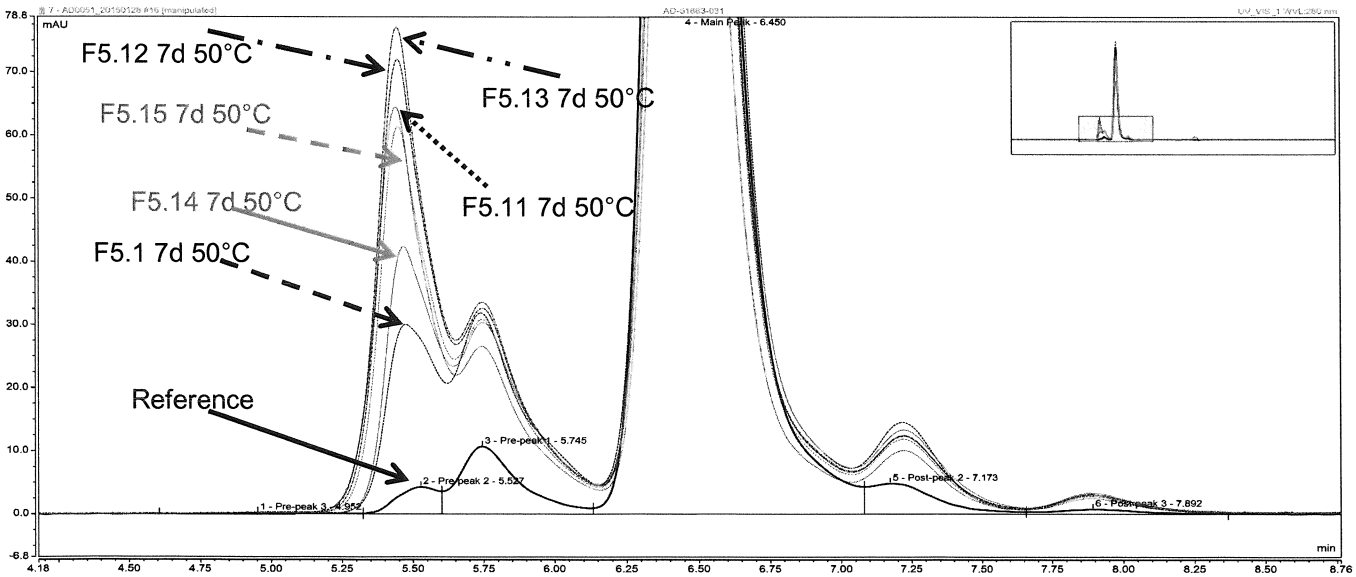
Add 7.10 g sodium phosphate dibasic, to a 1000mL beaker
Add 250.0 g sodium citrate tribasic
Add approximately 800mL Milli-Q water
Mix well and adjust pH to 6.0±0.2 with 5M phosphoric acid
Transfer the solution to a volumetric flask and fill to volume with Milli-Q water
Filter the solution through 0.2 µm filter.
Store the mobile phase buffer at room temperature for up to 1 month.

2. RESULTS

The stability of formulation F5.1 after 7 days at 50°C (which falls within the scope of claim 1), as measured by size exclusion chromatography (SEC) and hydrophobic interaction chromatography (HIC) is higher than the stability of formulations F5.11 to F5.15. As showed below, the % of main peak (%MP) is higher in F5.1 formulation as compared with F5.11 to F5.15 formulations, both when using SEC and HIC.

Sample Description	SEC			HIC		
	%Prepeaks	%MP	%Postpeaks	%Prepeaks	%MP	%Postpeaks
F5.1 7d 50°C	13.9	81.0	5.1	3.8	72.1	24.1
F5.11 7d 50°C	26.6	68.1	5.3	3.8	66.0	30.2
F5.12 7d 50°C	24.3	70.4	5.3	3.7	66.3	30.0
F5.13 7d 50°C	25.2	69.6	5.2	3.5	65.9	30.6
F5.14 7d 50°C	17.2	77.2	5.6	3.7	71.0	25.3
F5.15 7d 50°C	22.4	72.3	5.3	3.6	67.6	28.8

Size exclusion chromatography (SEC):



Hydrophobic interaction chromatography (HIC):