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LC/MS analysis of hydrophilic compounds in biological fluid by a polymer-based reversed-phase column

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Abstract

In this study, a polyhydroxymethacrylate-based reversed-phase column, Shodex ODP2 HP, was used to develop high-sensitive LC/MS method for the quantification of various drugs in the presence of protein. Comparison data using a silica-based reversed-phase column is also presented.

Presence of hydroxyl group in ODP2 HP's base material provides a unique characteristic to this column, which creates longer retention for hydrophilic compounds than generally available reversed-phase columns. In addition, the small pore size on the packed gel provides size exclusion effect. This works to remove potential interfering high molecular weight biomolecules such as proteins as a void, and thus helps the analysis of later-eluting low molecular weight drugs.

Statins are a group of drugs used to lower cholesterol level by inhibiting HMG-CoA reductase. It is essential to determine the concentration of statins in serum in order to monitor the drug's efficiency. A mixture of seven statins and BSA was used as a model to imitate the serum matrix sample during the method development. Seven statins were separated within 15 min. Sharp symmetrical peaks were obtained for all statins with minimum ion suppression.

Alkaline durability of Shodex ODP2 HP is especially beneficial for the analysis of basic drugs; as alkaline condition provides stronger retention for basic compounds. Moreover, the alkaline condition enhanced the size exclusion effect of removing interfering proteins, consequently reduced its ion suppression impact.

Comparison of Shodex ODP2 HP vs. Conventional ODS columns

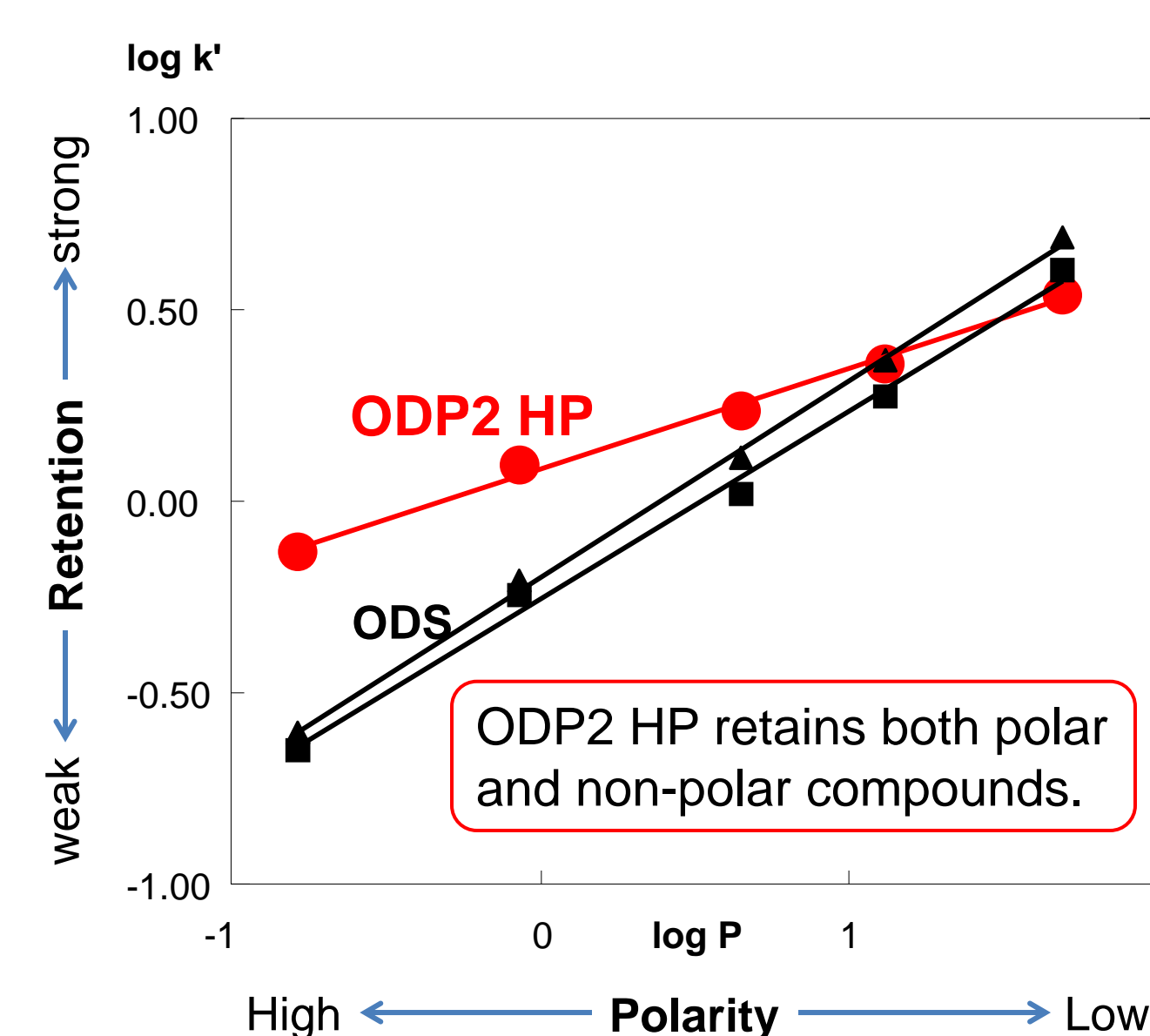
Column Specifications

	ODP2 HP	ODS
Base material	Polyhydroxymethacrylate	Silica
Functional Group	None	Octadecyl (C18)
Average Pore Size (Å)	40	100 – 120
Available pH	3 – 12	2 – 8

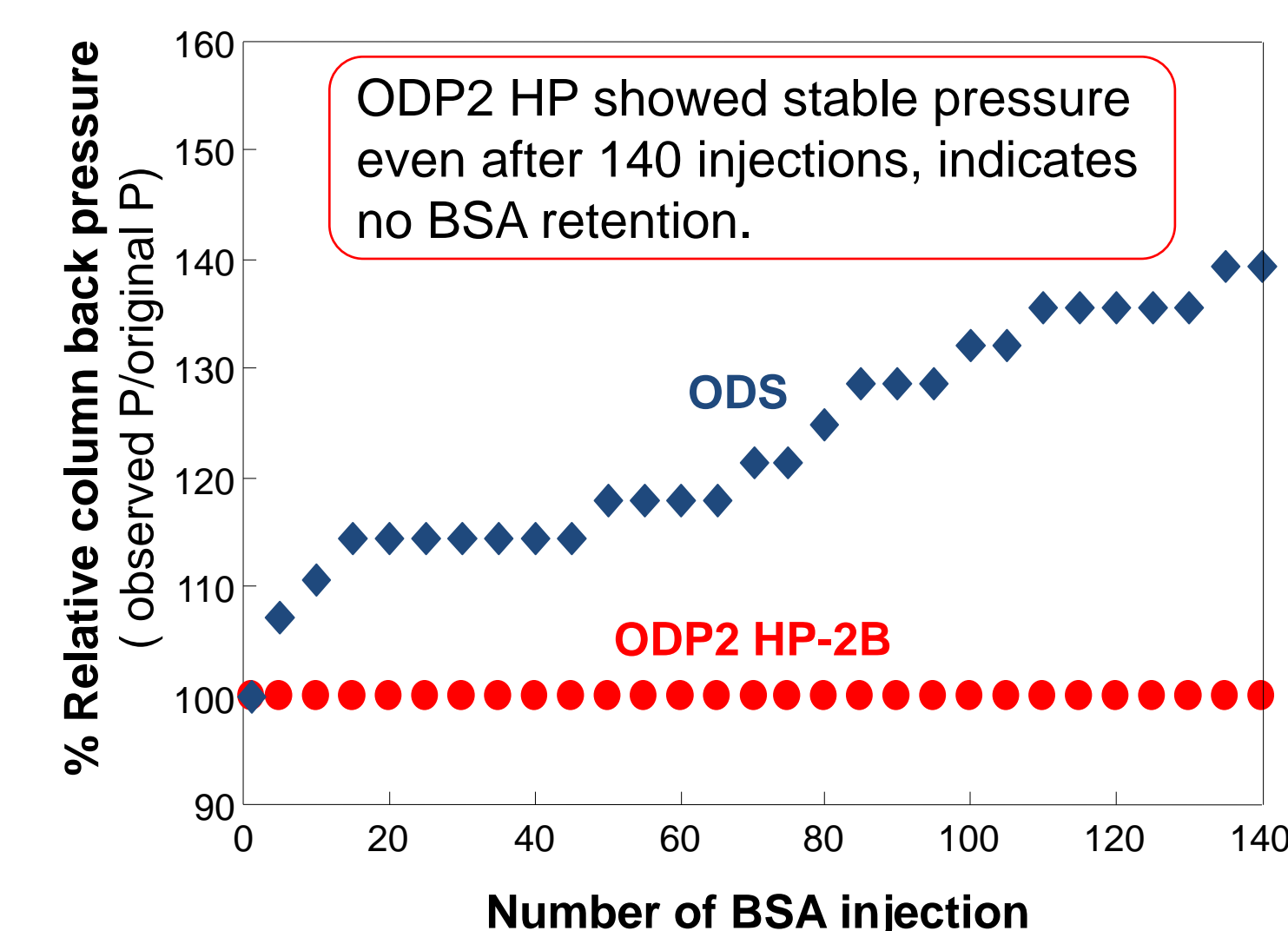
Both hydrophobic and hydrophilic molecules are retained while protein is not.

Protein easily adsorbed. Small retention of hydrophilic molecules.

Sample polarity and retention



Number of BSA injection and column back pressure



ODP2 HP series line up

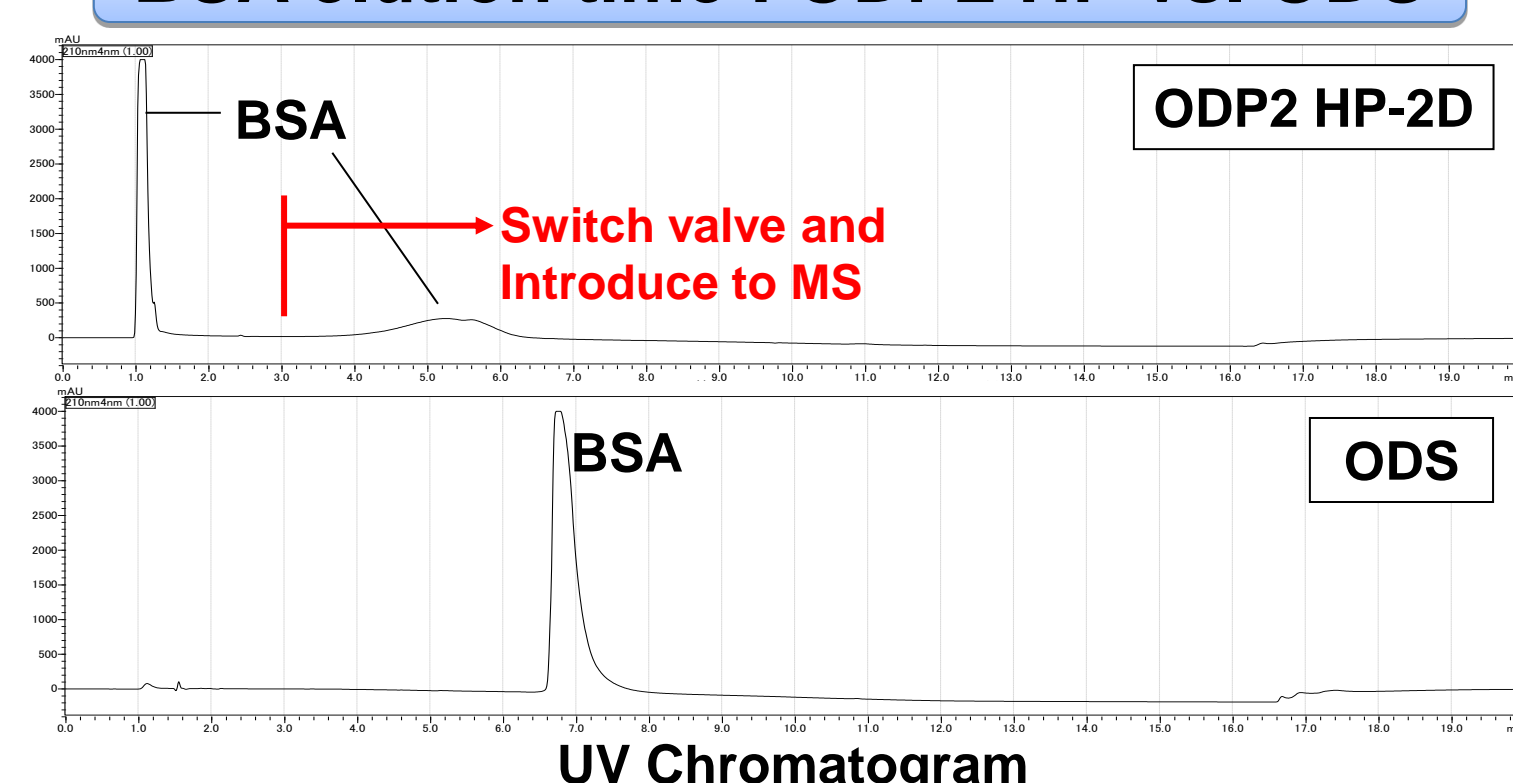
Product name	Plate Number (TP/Column)	Particle Size (µm)	Column Size (mm) I.D. x Length
ODP2 HP-4B	≥ 3,500	5	4.6 x 50
ODP2 HP-4D	≥ 13,000	5	4.6 x 150
ODP2 HP-4E	≥ 17,000	5	4.6 x 250
ODP2 HPG-4A (guard column)		5	4.6 x 10
ODP2 HP-2B	≥ 3,000	5	2.0 x 50
ODP2 HP-2D	≥ 7,000	5	2.0 x 150
ODP2 HPG-2A (guard column)		5	2.0 x 10

Suitable column dimensions for LC/MS



[Result 1] Analysis of statins

BSA elution time : ODP2 HP vs. ODS



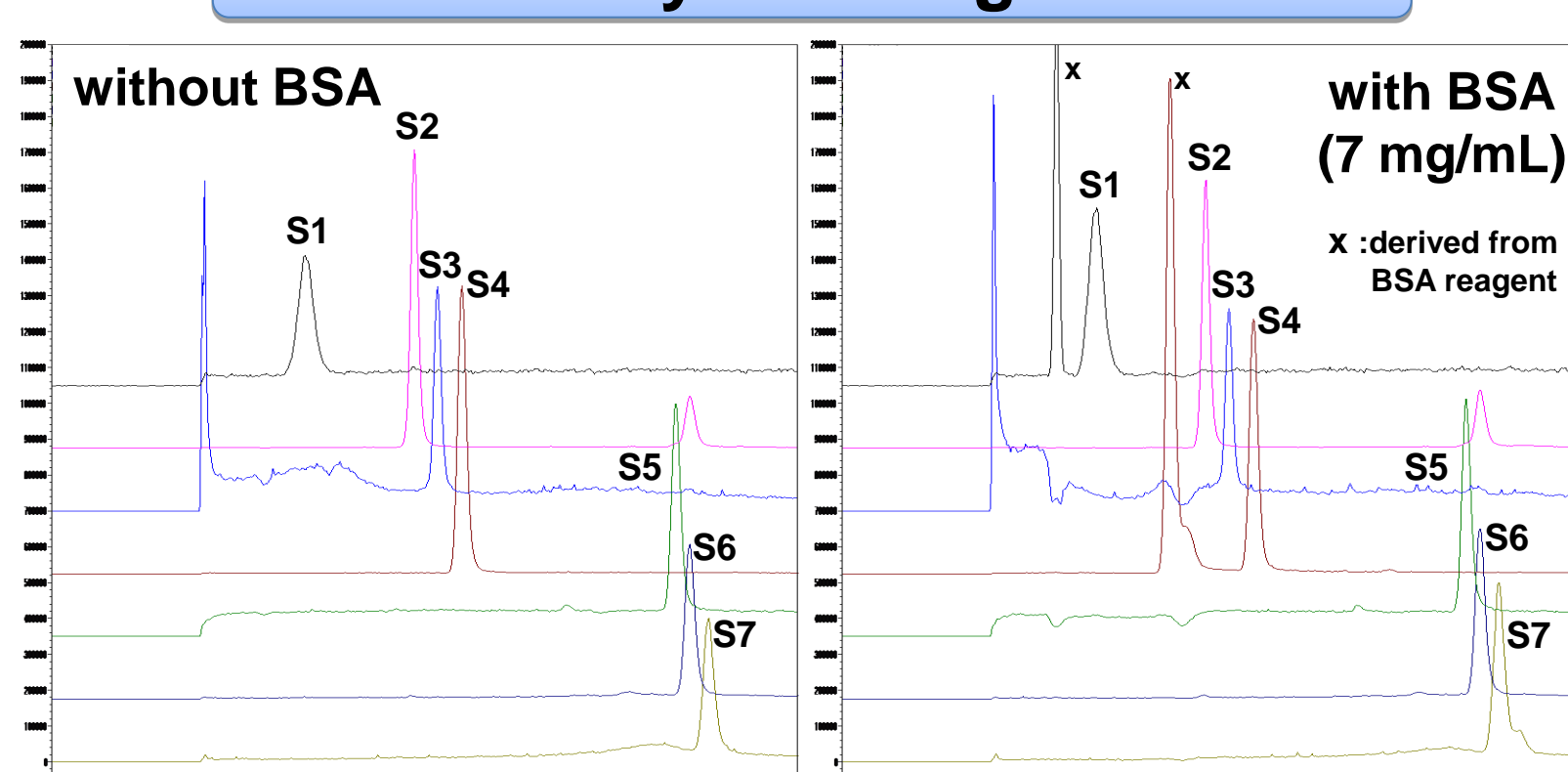
ODP2 HP-2D

- Most BSA eluted at V₀ and this portion was not going to be introduced to MS
- No column back pressure increase was observed even in the presence of BSA

ODS

- BSA is retained and BSA peak overlaps with analyte peaks
- Column back pressure increased due to partial adsorption of BSA
- BSA stained the ion source

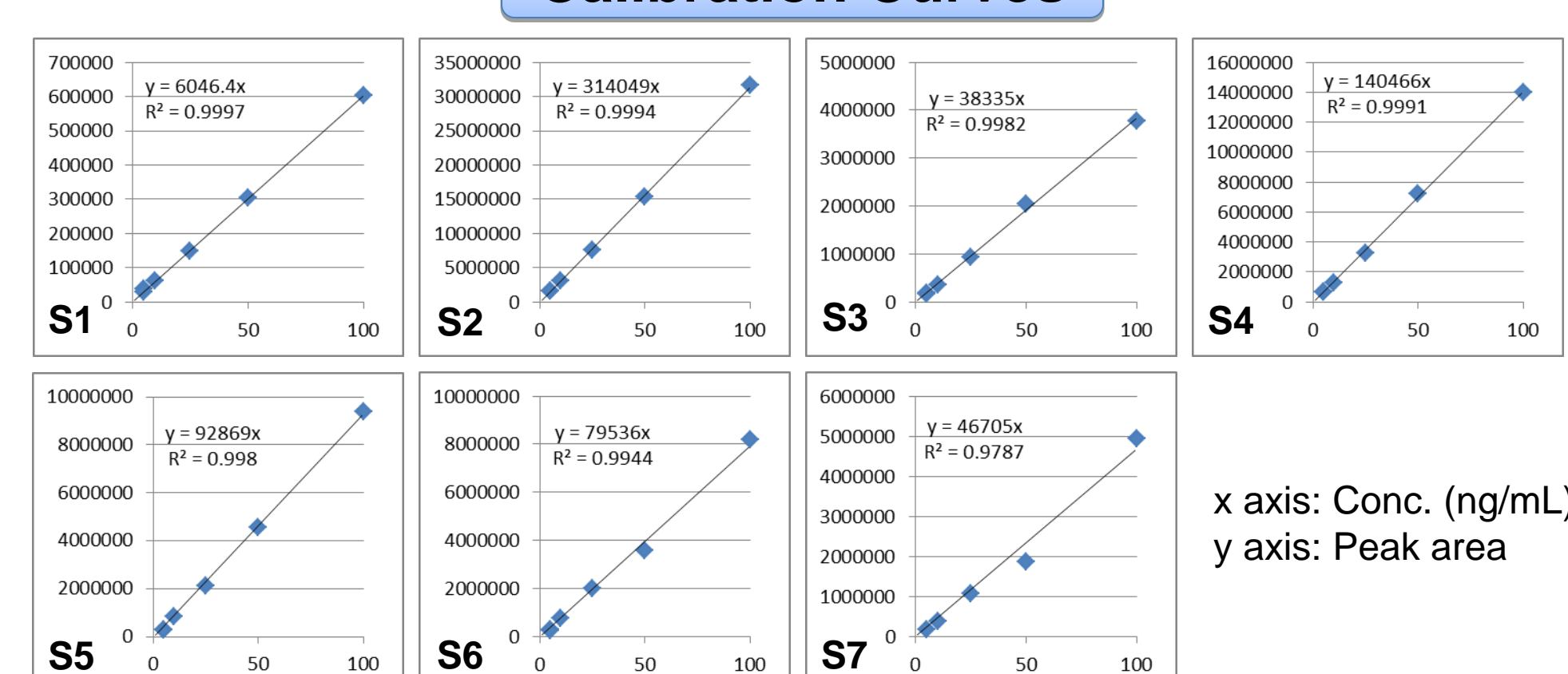
LC/MS analysis using ODP2 HP



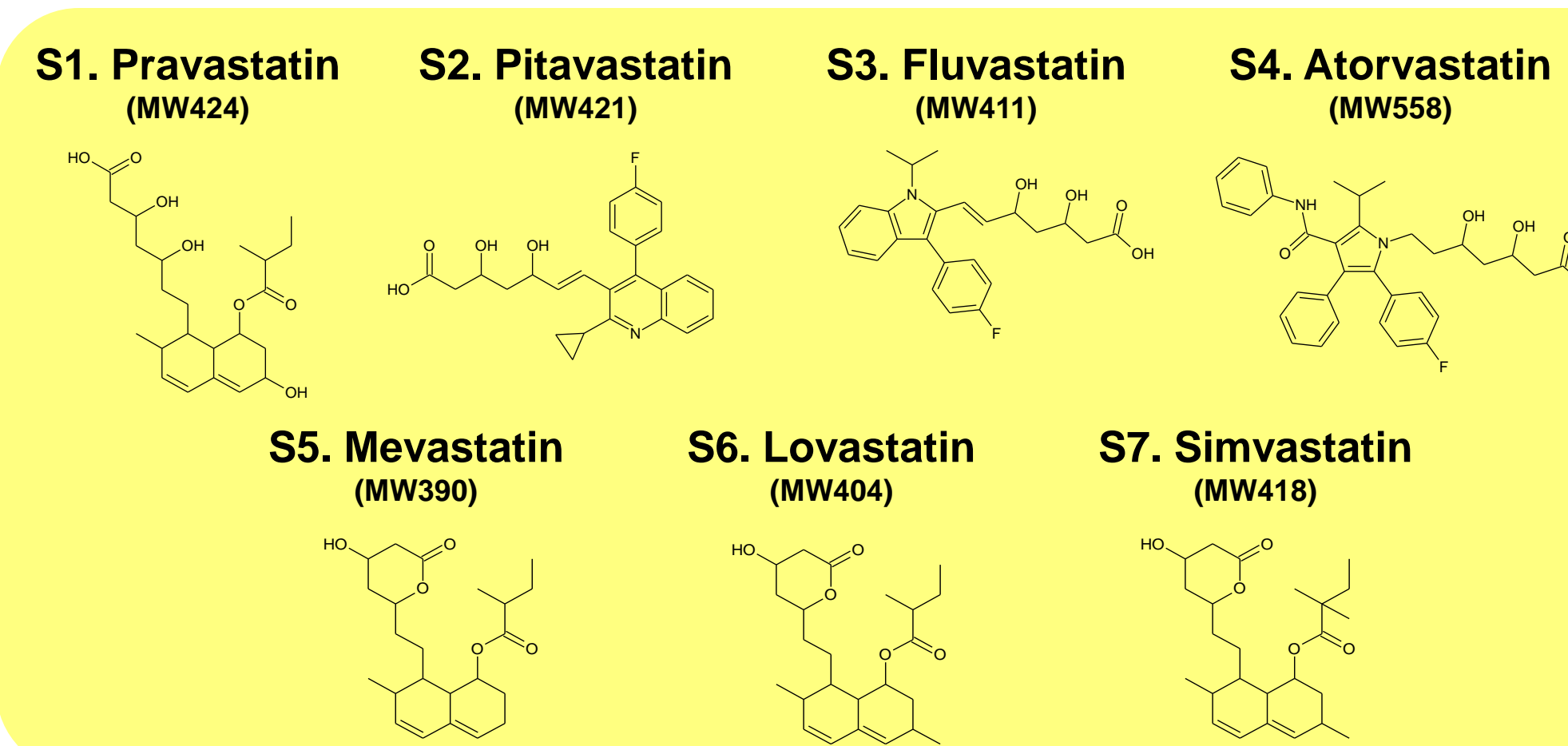
- Seven statins were separated within 15 min
- Good recovery rates were obtained

Statin	S1	S2	S3	S4	S5	S6	S7
CV(%) without BSA	3.8	1.7	1.2	2.1	5.9	8.4	9.4
CV(%) with BSA	5.3	4.0	1.9	5.4	9.0	10.1	13.7
Recovery rate(%)	126	88	84	86	90	94	103

Calibration Curves



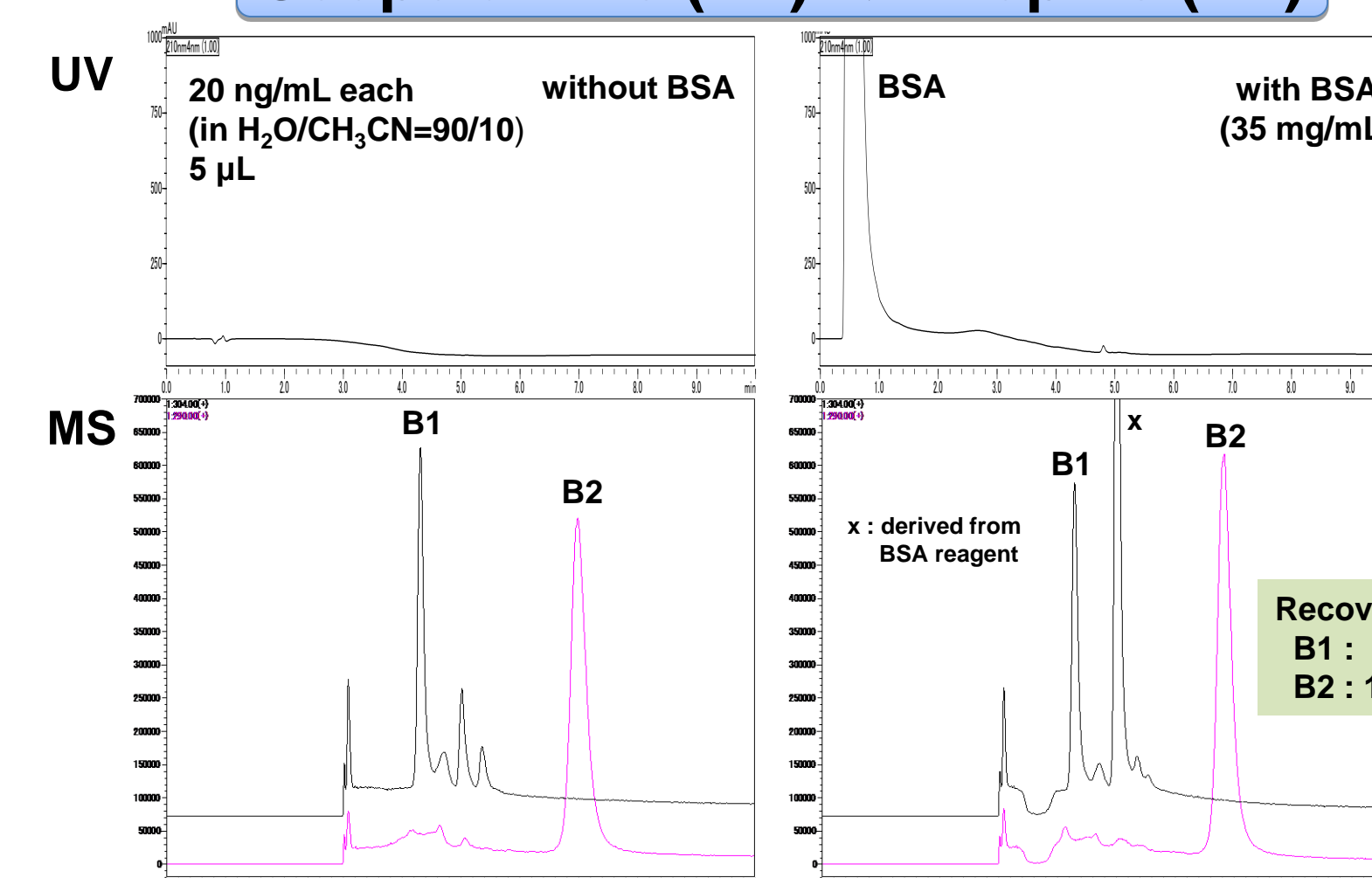
All statins showed high linearity in 5 - 100 ng/mL range.



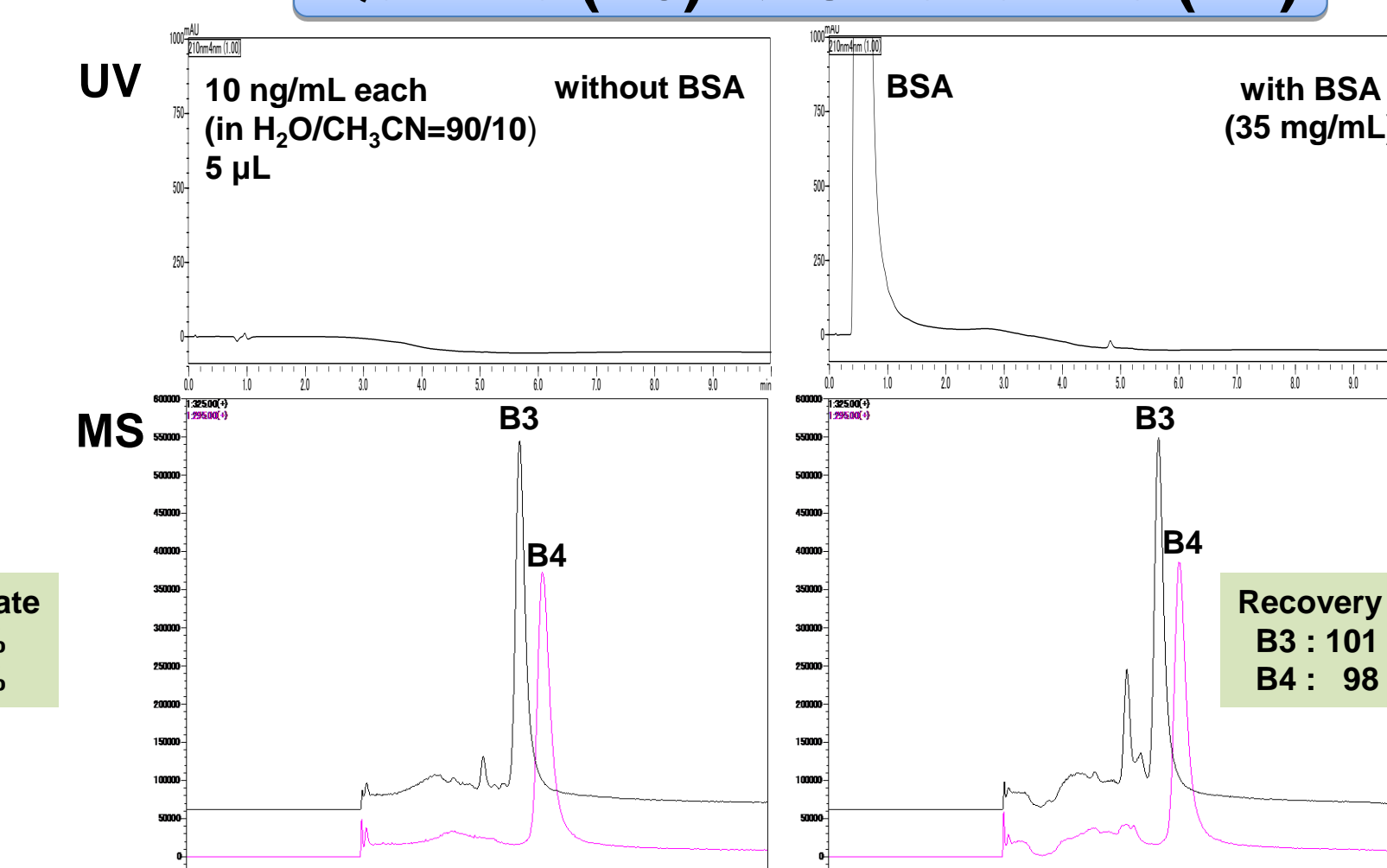
Column : 1. Shodex ODP2 HP-2D (2.0 mm I.D. x 150 mm)
2. ODS (2.0 mm I.D. x 150 mm)
Eluent : 1. (A) 10 mM HCOONH₄ (aq) pH 9.0/(B) CH₃CN
Linear gradient; (B%) 5% (0 min) → 50% (10-15 min)
2. (A) 10 mM HCOONH₄ (aq) pH 6.5/(B) CH₃CN
Linear gradient; (B%) 10% (0 min) → 85% (10-15 min)
Flow rate : 0.2 mL/min Column temp. : 30 °C
Instrument : Shimadzu Nexera / LCMS-8030
Detector : UV (210 nm), ESI-MS (SIM)
Sample : Statins 50 ng/mL each (in H₂O/CH₃CN=90/10)
Injection volume : 5 µL

[Result 2] Analysis of basic drugs under alkaline condition

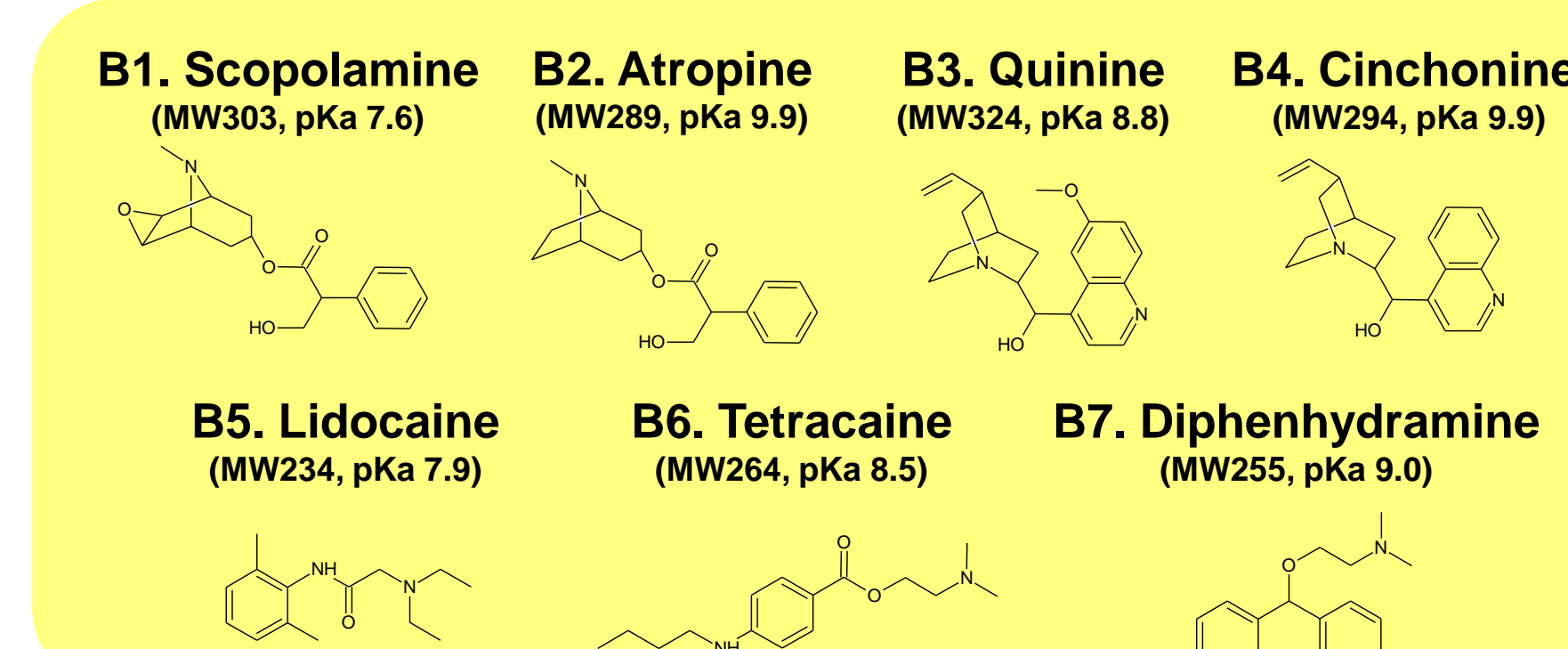
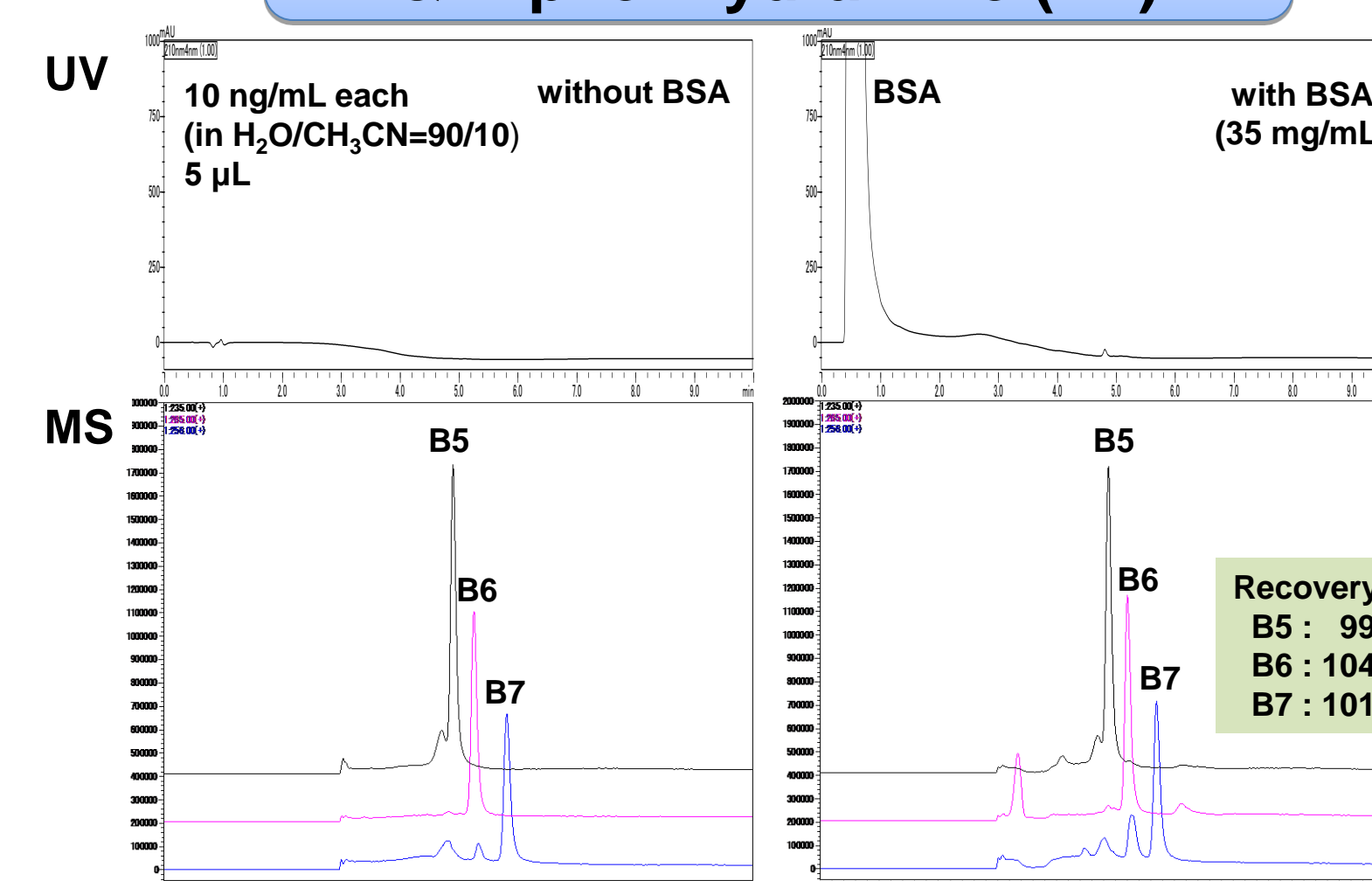
Scopolamine (B1) & Atropine (B2)



Quinine (B3) & Cinchonine (B4)



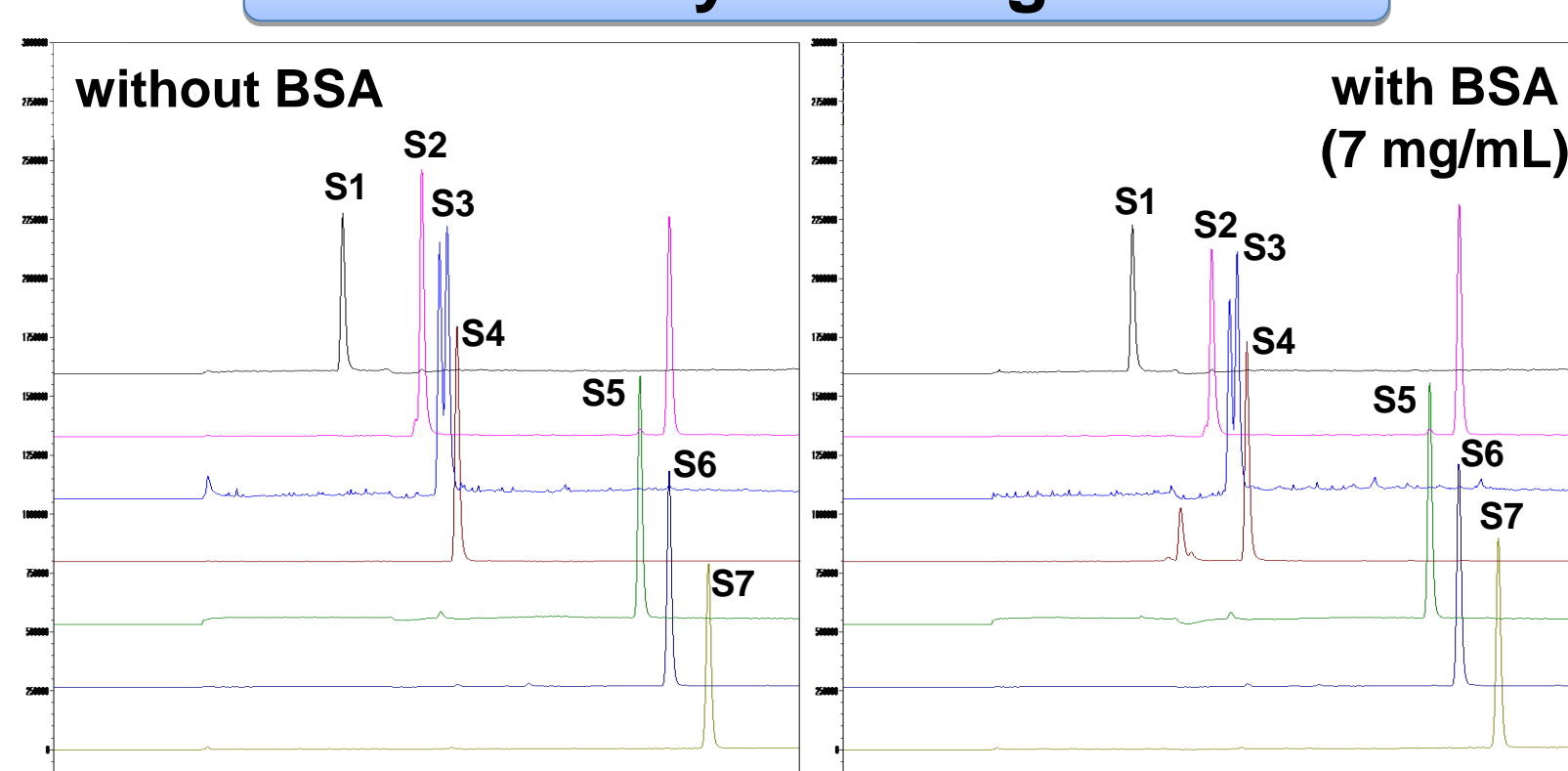
Lidocaine (B5), Tetracaine (B6) & Diphenhydramine (B7)



Column : Shodex ODP2 HP-2B (2.0 mm I.D. x 50 mm)
Eluent : (A) 0.05% NH₃ (aq) pH 11.0/(B) CH₃CN
Linear gradient; (B%) 5% (0 min) → 90% (5-10 min)
Flow rate : 0.2 mL/min Column temp. : 30 °C
Instrument : Shimadzu Nexera / LCMS-8030
Detector : UV (210 nm), ESI-MS (SIM)

- Under pH 11, BSA eluted completely at V₀, while all basic drugs were retained in ODP2 HP
- No decrease in analyte peak intensity was observed even in the presence of BSA
- All basic drugs were sensitively detected without experiencing the ion suppression by BSA

LC/MS analysis using an ODS



- Back pressure increased in the presence of BSA
Injection No. : 1st 7.6 MPa, 2nd 8.0 MPa, 3rd 8.3 MPa, 4th 8.5 MPa
- Pitavastatin's (S2) peak intensity decreased considerably in the presence of BSA

Statin	S1	S2	S3	S4	S5	S6	S7
CV(%) without BSA	7.1	6.0	4.3	5.0	5.5	8.6	12.1
CV(%) with BSA	5.9	1.2	6.4	2.8	2.8	4.4	7.8
Recovery rate(%)	88	67	85	86	99	108	123