

Development of Core Shell Particle with Large Pore for Separation of Peptides and Proteins

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Abstract

More than 10 kinds of core shell particle column have been available recently because core shell media offers significant improvements such as higher efficiency and lower pressure drop for existing HPLC operations without having to replace existing HPLC systems with UHPLC systems. Moreover, large molecules such peptides or proteins have been watched as medicine in recent years. Silica gel with 30 nm pores has generally been used for separation of proteins.

In this study, a 2.6 μm core-shell silica with a non-porous core approximately 1.6 μm in diameter and a superficially porous layer of 0.5 μm and 30nm pore was developed. As a novel bonding chemistry, hexa-functional C18 reagent with two sets of trichlorosilane was applied. It is considered that this reagent makes acidic stability high because of six positions of siloxane at most. Acidic stability was evaluated under 0.1 % formic acid solution/acetonitrile as a typical LC/MS mobile phase condition at 70 degree Celsius. It was confirmed that the hexa-functional C18 was stable for more than 1000 hours. The developed materials bonded with hexa-functional C18, C8 and C4 were evaluated to separate not only standards of peptides and proteins but also tryptic digest of a protein using UV and Mass spectrometry detectors.

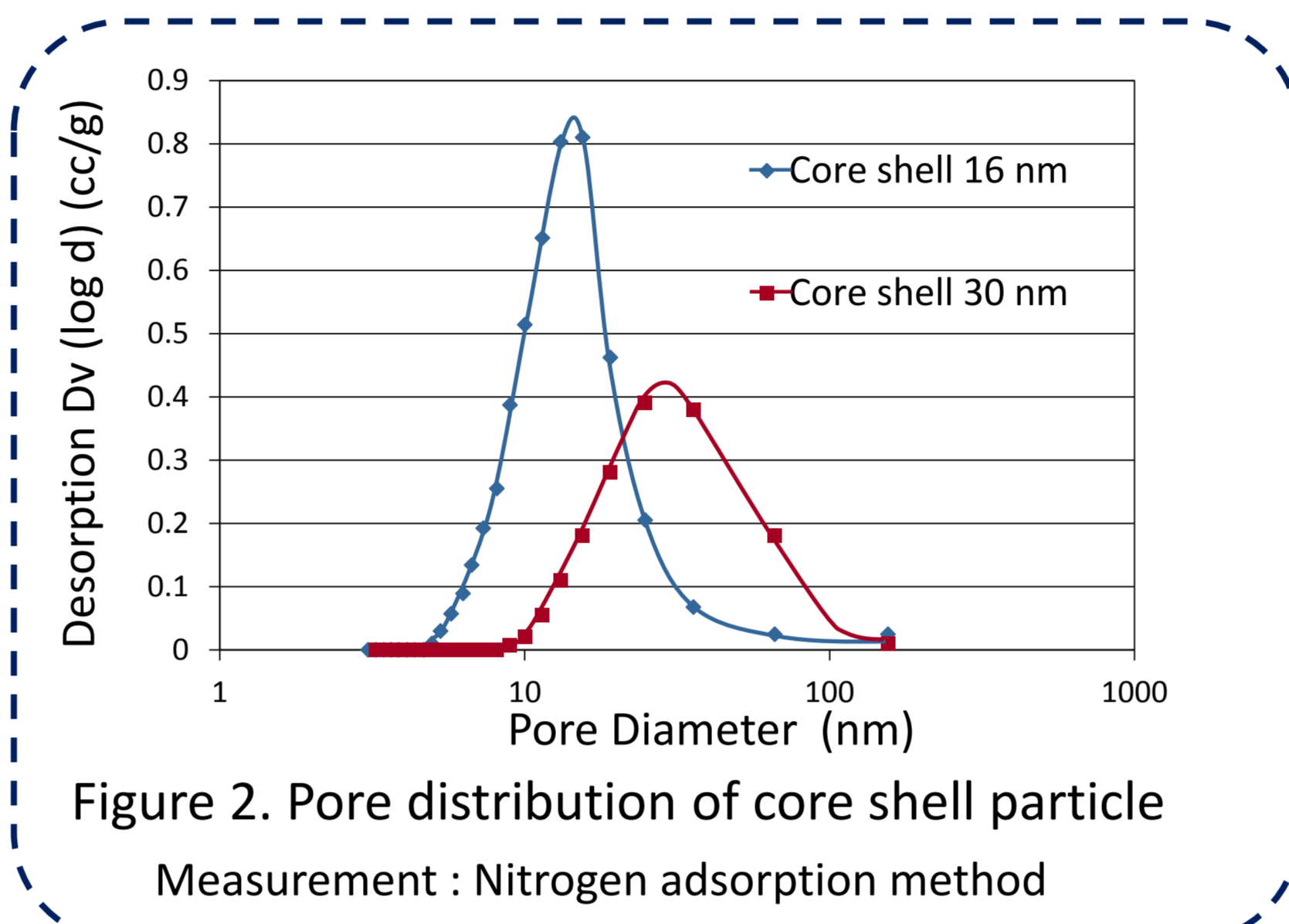
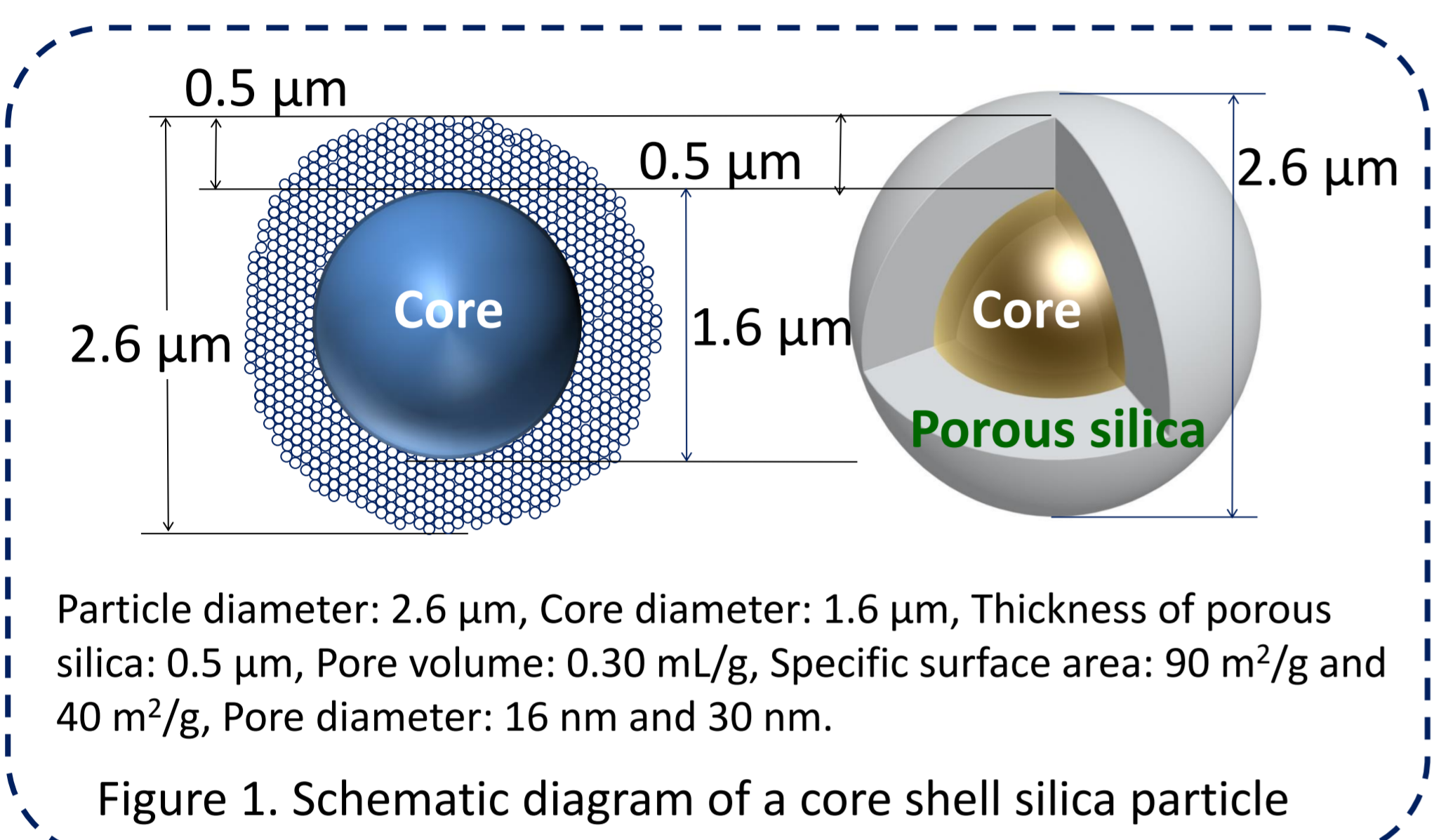
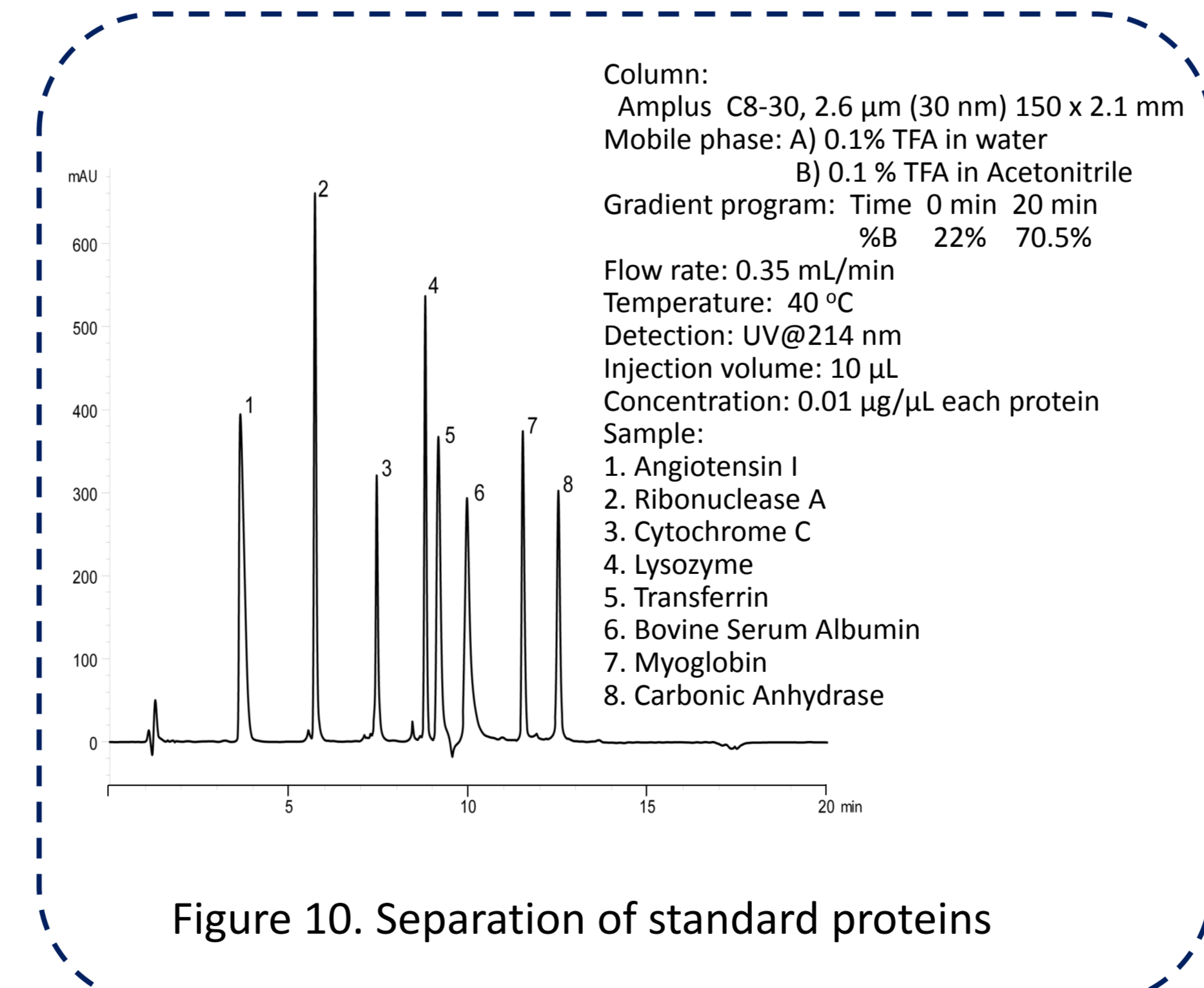
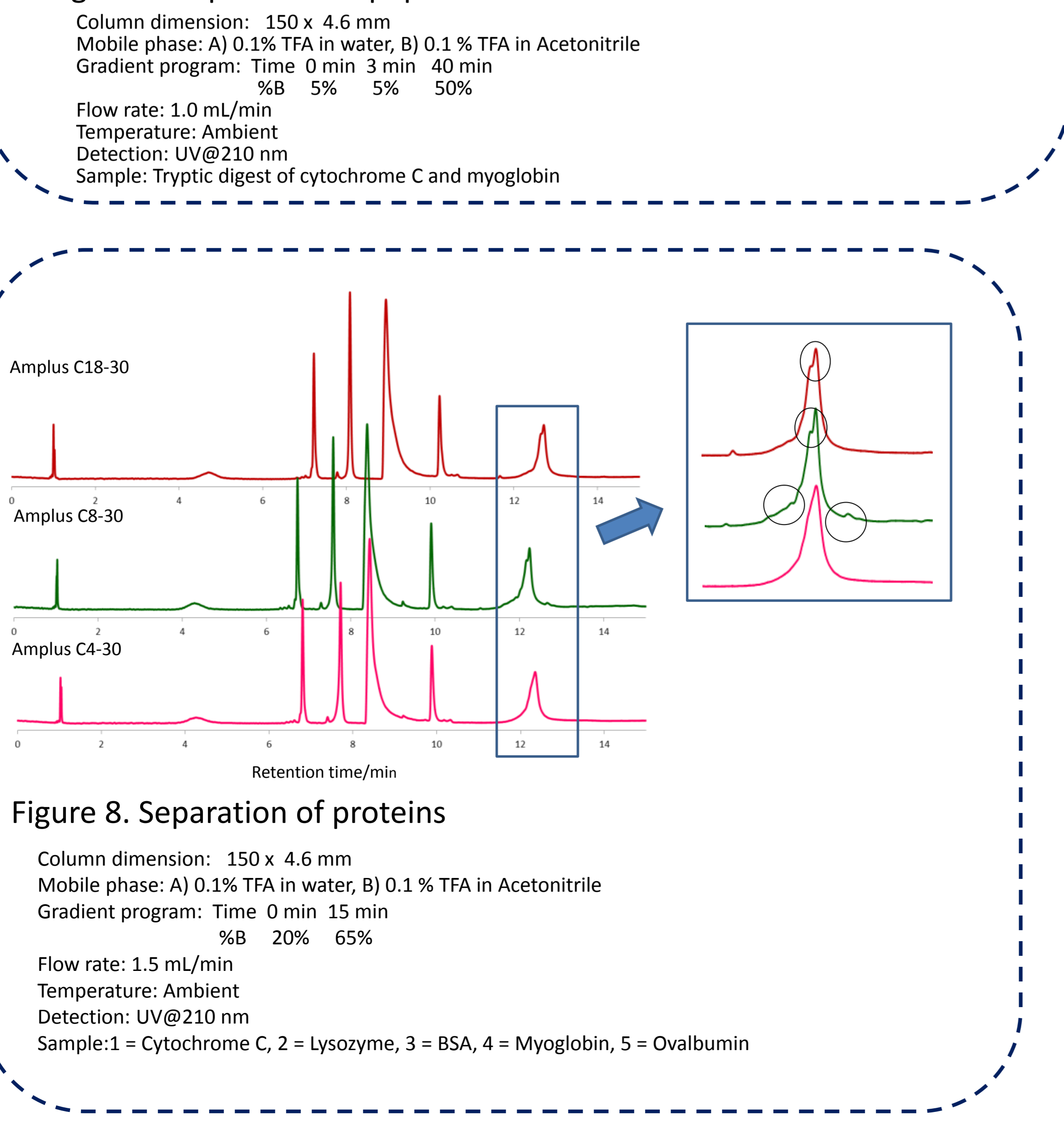
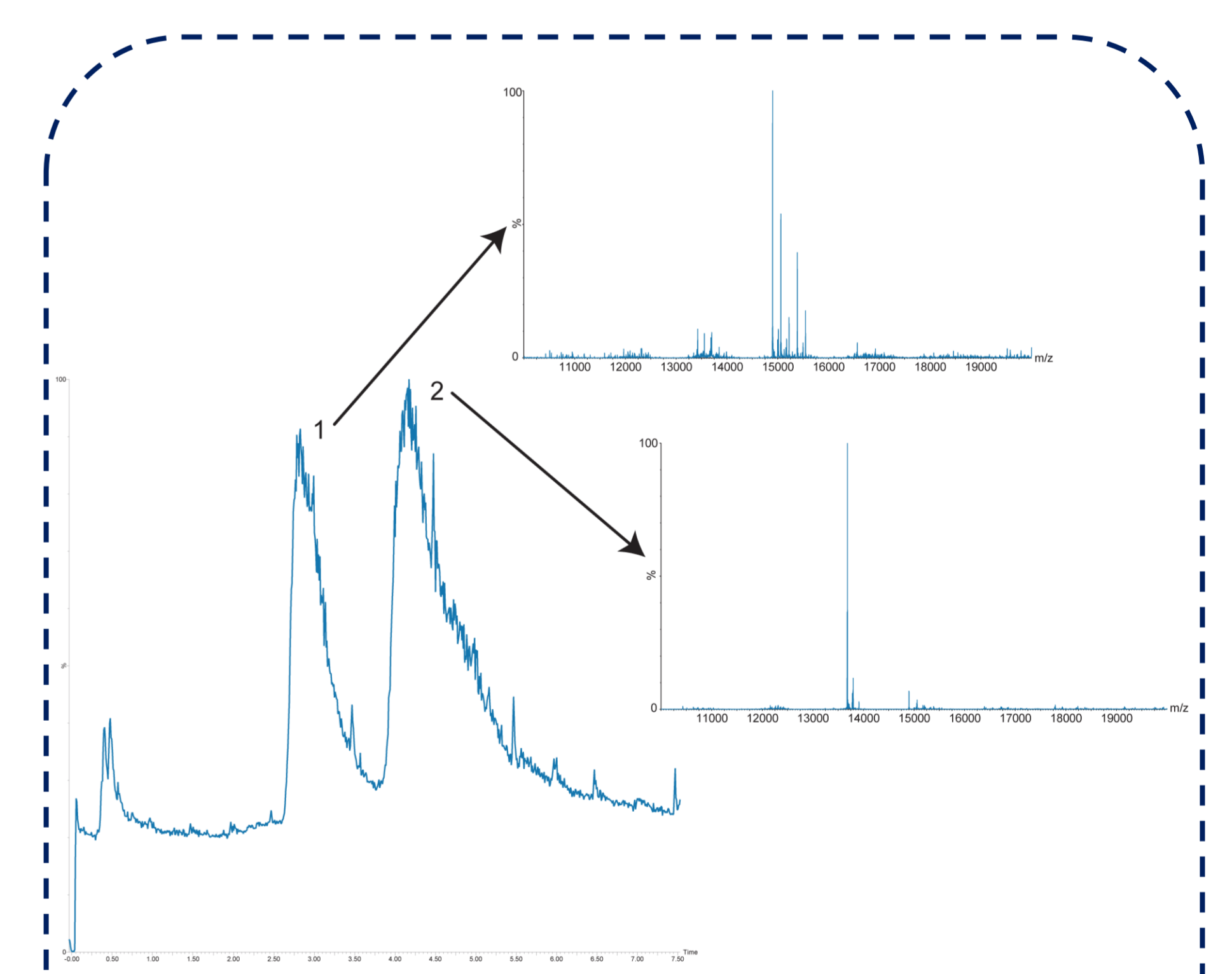
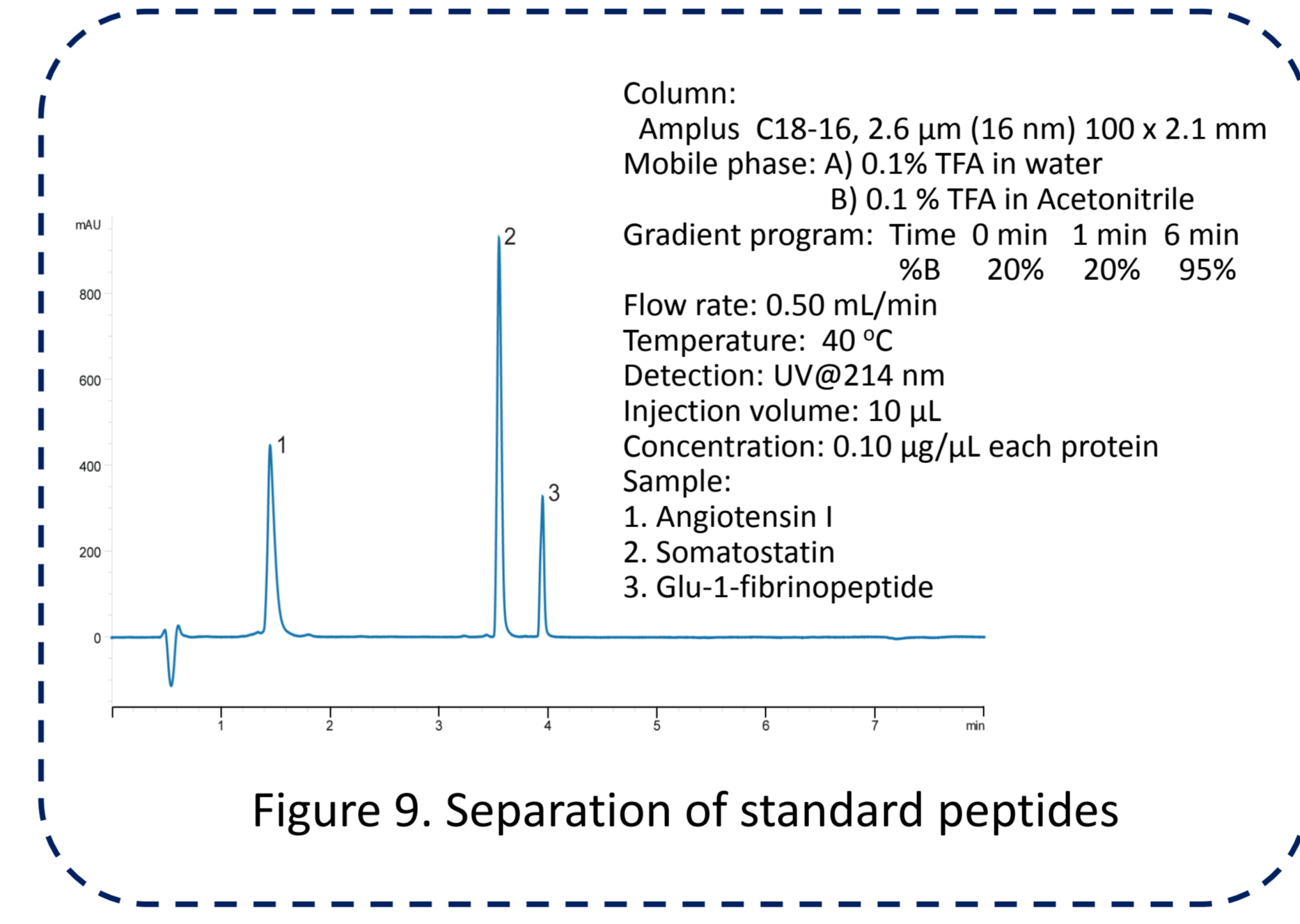
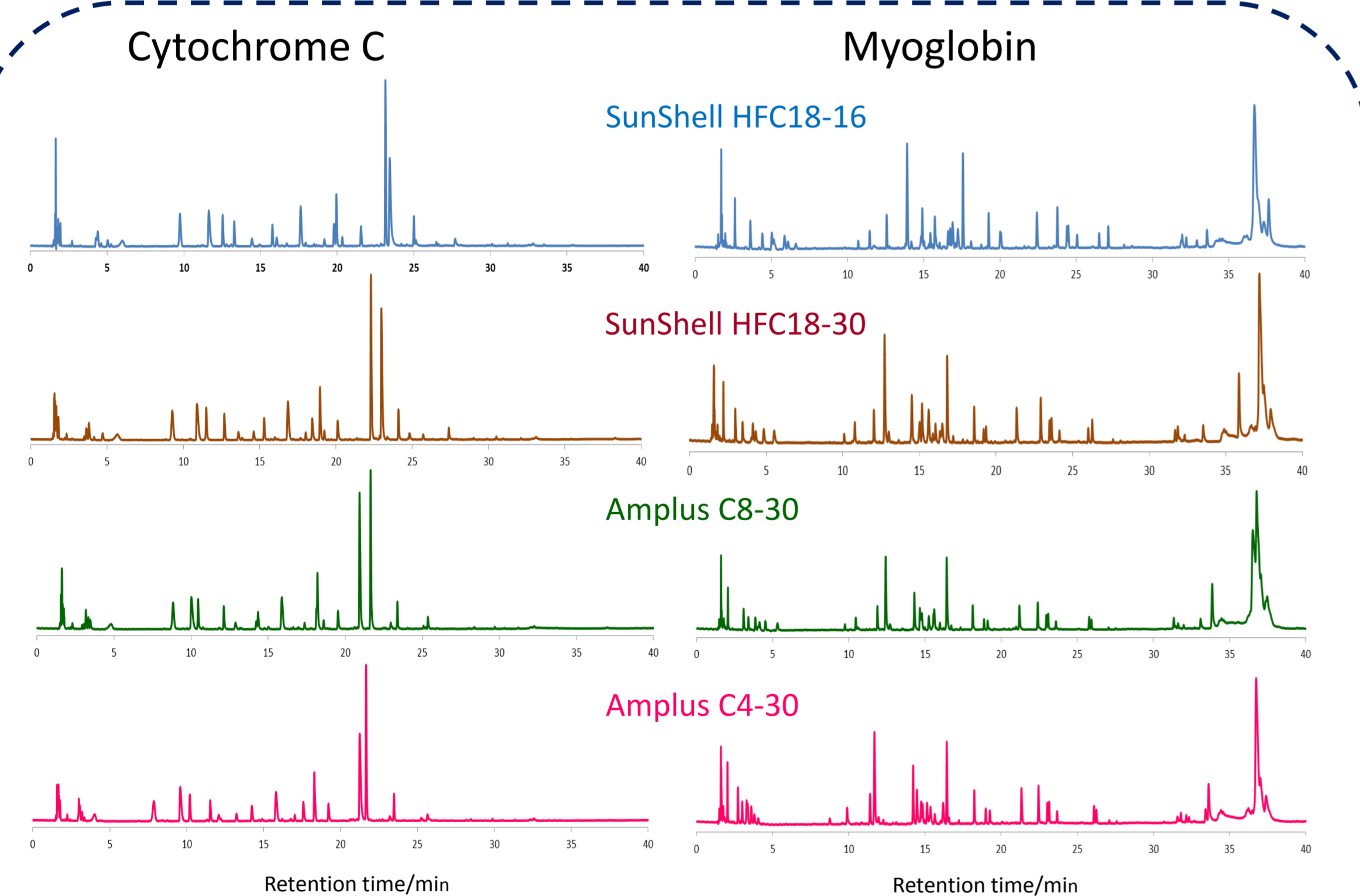
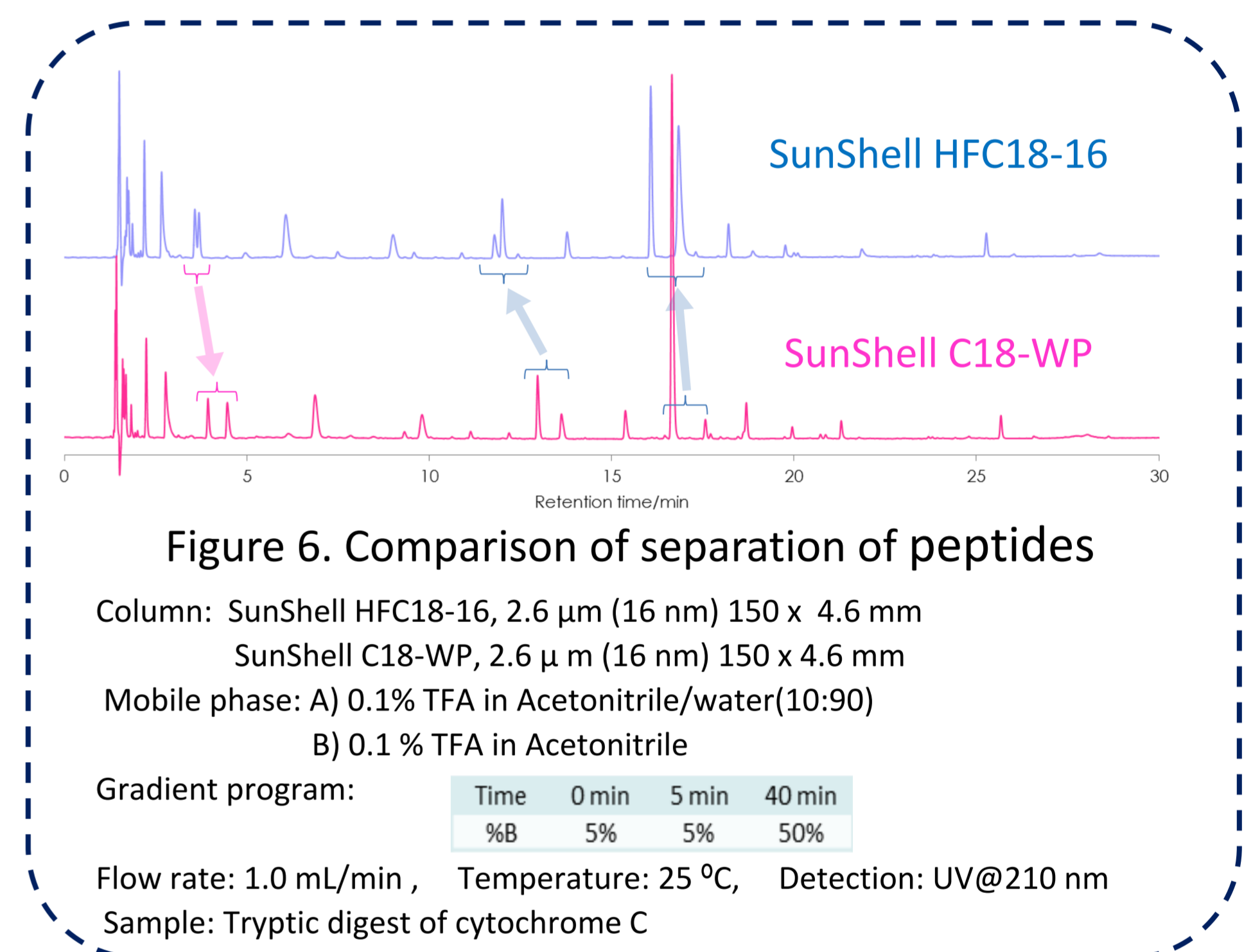
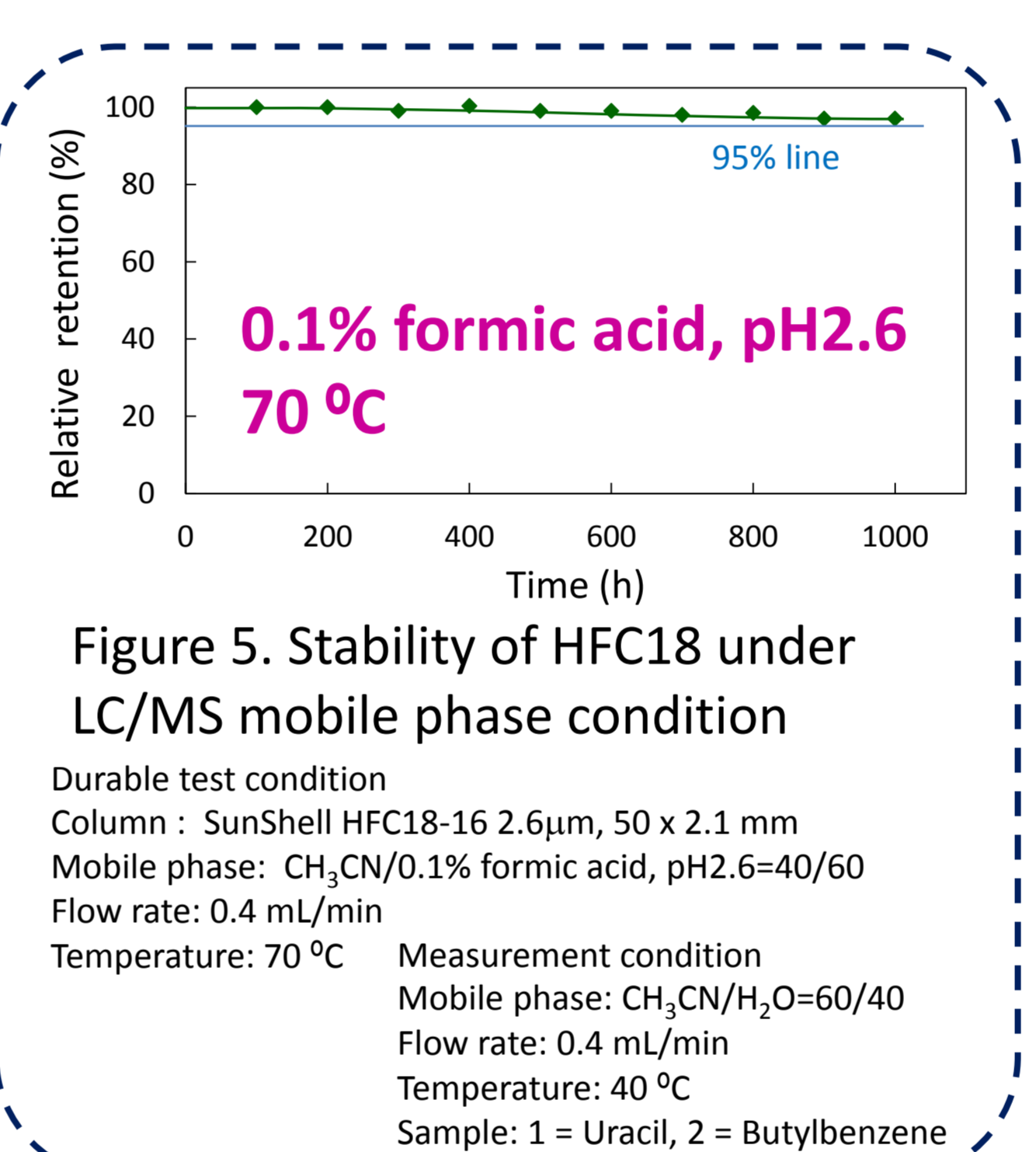
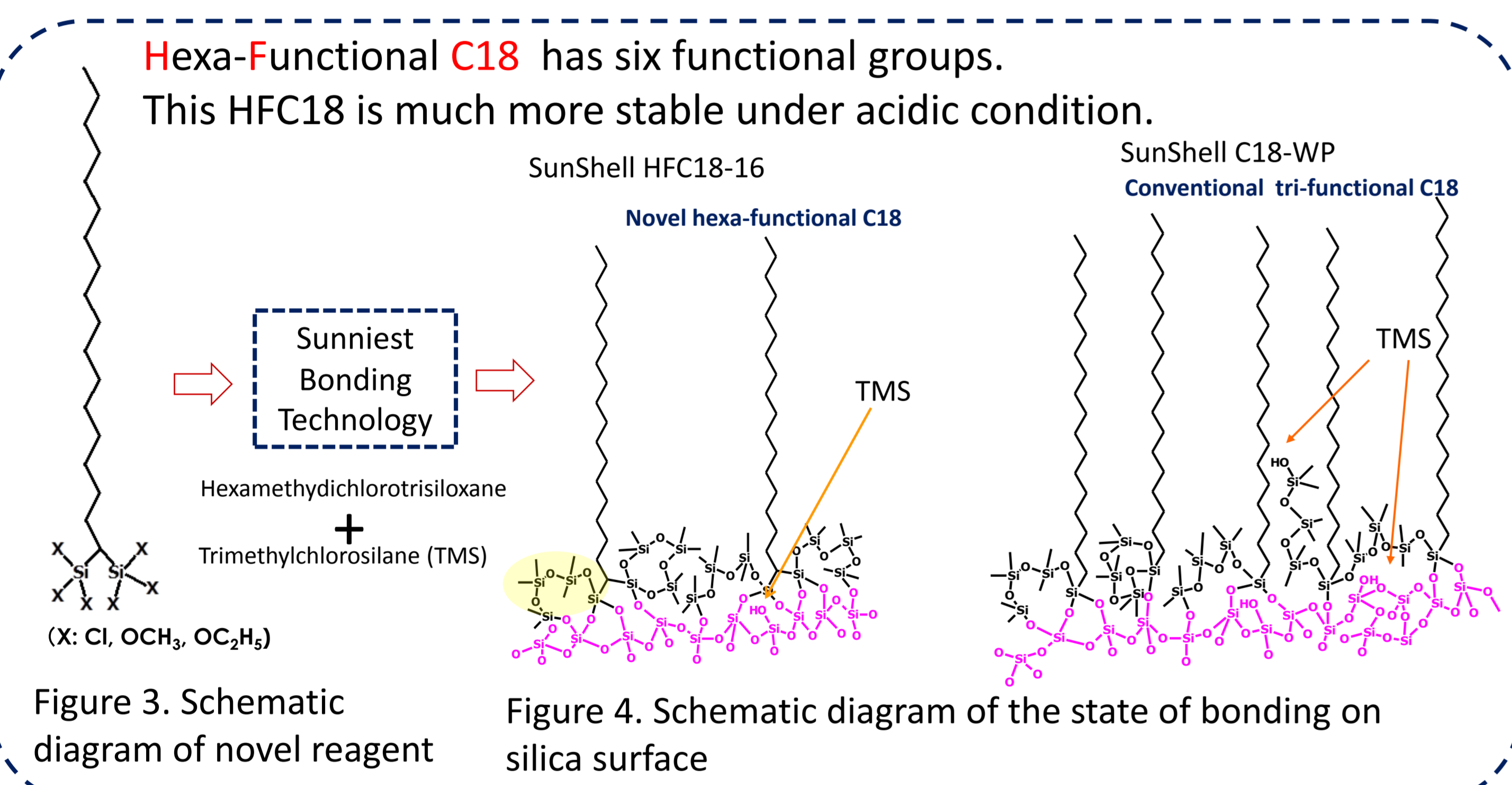


Table 1. Pore distribution of core shell particle

	Pore diameter	Surface area	Carbon loading	Ligand Surface coverage	End-capping
SunShell C18-WP	16 nm	90 m^2/g	5%	2.5 $\mu\text{mol}/\text{m}^2$	Yes
SunShell HFC18-16	16 nm	90 m^2/g	2.5%	1.2 $\mu\text{mol}/\text{m}^2$	Yes
SunShell HFC18-30	30 nm	40 m^2/g	1.3%	1.2 $\mu\text{mol}/\text{m}^2$	Yes
Amplus C18-16	16 nm	90 m^2/g	2.5%	1.2 $\mu\text{mol}/\text{m}^2$	Yes
Amplus C18-30	30 nm	40 m^2/g	1.3%	1.2 $\mu\text{mol}/\text{m}^2$	Yes
Amplus C8-30	30 nm	40 m^2/g	1.2%	2.5 $\mu\text{mol}/\text{m}^2$	Yes
Amplus C4-30	30 nm	40 m^2/g	0.9%	3 $\mu\text{mol}/\text{m}^2$	Yes



Conclusion

- *Regarding the pore size distribution, 30nm core shell particle was wider than 16 nm one.
- *A novel Hexa-functional C18 phase showed not only high acidic stability for more than 1000 hours under typical LC/MS mobile phase condition but also different selectivity of peptides from a conventional tri-functional C18.
- *30 nm C8 phase showed the best separation of ovalbumin in the three kinds of 30 nm phase.

