

Chemical Metrology Laboratory Instruction on Sample Analysis

1. Schedule

Collection of samples: 27 Jul – 03 Aug 2017

Deadline for submission of results: 03 Sep 2017

2. Objective

To measure the intensities of peptide standard (VHLTPE) and its isotopic standard (VHL*TPE) in the samples provided using the same system offered in this tender.

3. Samples

The samples consist of peptides that have been diluted with water. Each vendor will be provided with 8 x 2.0 mL disposable centrifuge tubes containing different solutions. The tubes are labelled as: 1) CB1-CB4, 2) SB1-SB2, 3) STD and 4) ISTD. The samples must be stored in a freezer (<-20°C) upon receipt until analysis. Exposure to direct intense light and ultraviolet radiation should be avoided. The information of all samples is as follows:

CB1-CB4: Calibration Blends 1 to 4.

SB1-SB2: Sample Blends 1 and 2.

STD: Peptide standard solution (~ 1 µg/mL) for LC and MS method development ONLY.

ISTD: Isotope standard solution (~ 1 µg/mL) for LC and MS method development ONLY.

4. Collection

To ensure that the integrity of the samples is not compromised, it is strongly recommended to bring along a foam box containing dry ice for storage of samples during collection.

4. Instructions for Analysis

The instrument should be tuned according to the instrument specifications. The samples (CB1-CB4 and SB1-SB2) must be analysed directly (without any diluting or concentrating process) in 1 sequence as shown below:

Table 1. Sequence of injection.

Injection Sequence	Peak Area (695.4 → 350.2)	Peak Area (702.5 → 357.2)
Blk-1		
CB1-1		
CB2-1		
CB3-1		
CB4-1		

Injection Sequence	Peak Area (695.4 → 350.2)	Peak Area (702.5 → 357.2)
SB1-1		
SB2-1		
SB2-2		
SB1-2		
CB4-2		
CB3-2		
CB2-2		
CB1-2		
Blk-2		
CB1-3		
CB2-3		
CB3-3		
CB4-3		
SB1-3		
SB2-3		
SB2-4		
SB1-4		
CB4-4		
CB3-4		
CB2-4		
CB1-4		
Blk-3		

By using this sequence, each sample (CB and SB) should be injected 4 times. For each CB and SB, both VHLTPE and VHL*TPE should be monitored. The monitored masses must be those listed in the Table 2 (the 0.1 digit of each mass may vary).

Table 2. MRM Transition Masses.

Analyte	Parent Ion (M+H) ⁺	Daughter Ion
VHLTPE	695.4	350.2
VHL*TPE	702.5	357.2

Recommended LC parameters (Vendors can choose to use their own optimised parameters and column for run)

Column: Zorbax Eclipse Plus C18, 2.1 x 150 mm, 3.5 µm
Mobile Phase A: 0.1% Formic Acid in Water
Mobile Phase B: Acetonitrile
Flow: 0.3 mL/ min
Pump B Concentration: 5%-90% gradient
Injection Volume: 10 µL
Note: Equivalent condition and column are allowed.

5. Reporting of Results

The raw data should be exported into a Microsoft Excel file with the same sample sequence as listed in Table 1. The data must include the integration areas of the peaks of interest (MRM 695.4 → 350.2) and the internal standard (MRM 702.5 → 357.2) reported in the same format as Table 1. The Microsoft Excel file, the instrument operating parameters and tuning file should be submitted **not later than 2 weeks from the tender closing date**. Results should be submitted *via* email to Dr Wong Lingkai at Wong_Lingkai@hsa.gov.sg

Note 1: The results must fulfill the following two requirements for further evaluation of the tender.

- a) The injection relative standard deviation (RSD) of the isotope peak area ratio (VHLTPE/VHL*TPE) of each calibration blend or sample blend must be below 5%.
- b) The Calibration Curve must have a good-of-fit for linear regression (R^2) better than 0.995

Note 2: The concentration of each sample, the injection RSD, and the linearity of the calibration curve will be calculated by Chemical Metrology Laboratory using a validated Microsoft Excel template.

6. Safety and Handling

Treat the material as hazardous substance. Use appropriate work practices when handling the material, in order to avoid skin or eye contact or ingestion. Disposal of any discarded samples should be in accordance with any applicable waste management regulations in Singapore.