CHAPTER III

EXPERIMENTAL

3.1 Materials

1. Polyoxyethylene alkylamine standard

   TERWET 3780 ; Huntsman Co.,Ltd.

   RP II ; Wisca Co.,Ltd.

2. Herbicides

   Round Up ; Monsanto (Thailand) Co.,Ltd.

   Glyfosate 48 ; S and P Formulater Co.,Ltd.

   Margin 48 ; Acco (Thailand) Co.,Ltd.

3. Acetone

   Commercial grade; J.T. Baker

4. Acetonitrile

   Solvent grade; J.T. Baker

5. Methyl alcohol

   Solvent grade; J.T. Baker

5. Ethyl alcohol

   Solvent grade; J.T. Baker

6. Tetrahydrofuran

   HPLC grade; Labscan.

7. 2,5-Dihydroxybenzoic acid

   Aldrich

8. Dithranol

   Aldrich

9. α-cyano-4-hydroxycinamic acid

   Sigma

10. 2-(4-Hydroxyphenylazo)-benzoic acid

    Aldrich

11. Indole acrylic acid

    Sigma

12. *all-trans* Retinoic cid

    Sigma

13. Angiotensin II

    Sigma

14. Neurotensin

    Sigma

3.2 Apparatus and Instruments

1. Matrix-Assisted Laser Desorption Ionization Mass spectrometer (MALDI-MS)

   BIFLEX, BRUKER

2. Vortex mixer

   Vorttex-genie No.2, Scientific Industries

3. Multichannel probe

   BRUKER

4. Autopipette

   Pipetman, Gilson

5. Pipette tip

6. Eppendorf

3.3 Procedure

1. Condition for the analysis of polyoxyethylene alkylamine

- Target preparation
- Types of matrix
- Analyte-to-matrix ratio
- Precision

Sample preparation

Polymer sample (polyoxyethylene alkylamine(TERWET 3780); surfactant) for MALDI analysis was prepared by combining the surfactant which was dissolved in deionized water and various loading samples, various matrix solutions and various ratios of analyte to matrix.

Target preparation

Two methods for sample preparation were carried out.

1) Dried droplet method – sample and matrix were mixed and vortexed. Then a mixture of 1.0 µl was deposited on multiprobe and allowed to dry.
2) Thin layer method – The matrix solution was deposited on a multiprobe and allowed to dry and form a microcrystal layer. A sample solution containing analyte was added to the top of the matrix layer and dry. The matrix was droped again on the top of sample layer and allowed to dry.

Types of matrix

The following types of matrices in this experiment were separately used: 2,5-dihydroxybenzoic acid (DHB), dithranol, α-cyano-4-hydroxycinnamic acid (CCA), 2-(4-hydroxyphenylazo)-benzoic acid (HABA), indole acrylic acid (IAA), *all-trans* retinoic cid (RTA). Sample solution (5% w/w) and matrices were mixed (ratio of 1:225) and vortexed.

- 10 mg/ml DHB was prepared in acetone
- 10 mg/ml dithranol was prepared in THF

- 10 mg/ml CCA was prepared in ethanol/acetonitrile (50:50, 0.1% (V/V) TFA)
- 10 mg/ml HABA was prepared in THF
- 10 mg/ml IAA was prepared in acetone
- 10 mg/ml RTA was prepared in THF

Analyte-to-matrix ratio

The amount ratio of analyte solution (5 % w/w) to matrix were varied

- 1:160
- 1:180
- 1:200
- 1:220
- 1:240
- 1:260
- 1:280

Precision

Sample solution (5% w/w) and matrix were mixed (ratio of 1:225) and vortexed. Then a mixture of 0.5 and 1.0 µL was deposited on a multiprobe and allowed to dry. Finally, the multiprobe was taken into MALDI MS and bombarded with laser powder.

2. Quantification of polyoxyethylene alkylamine
   - Calibration curve used neurotensin as internal standard.
   - Accuracy
   - Quantification of polyoxyethylene alkylamine from herbicides.

Sample preparation

Prepare 10% (w/w) the standard (TERWET 3780 and RP II) in deionized water to be stock solution and dilute stock solution to concentration 10.00, 5.00, 2.50, 2.00, 1.00 and 0.50 % (w/w). Then standard solution and matrix solution were mixed with suitable type of matrix and ratio of analyte to matrix. And the internal standard mixture was prepared by mixing neurotensin which was dissolved in water (1mg/mL). Finally, mixture of standard and 1.0 µL of internal standard solution were mixed.

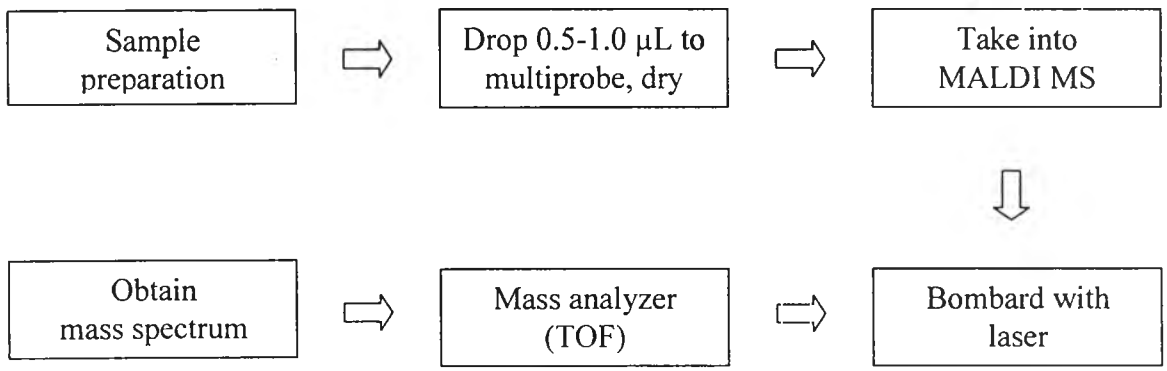
The mixture of standard and internal standard of 0.5 µL was deposited on a multiprobe and allowed to dry. Then the multiprobe was taken into MALDI MS and bombarded with laser powder. Calibration curve was created by plotting the concentration of standards and signal from mass spectra of standards to signal of internal standard.

For quantification of polyoxyethylene alkylamine from herbicides, the mixture was prepared like the mixture of standard but ratios of dissolving herbicides in water were 1:1, 1:2, 1:3, 1:4 and 1:5.

The range of laser power was used in this experiment were 240-280 µJ.

MALDI-TOF MS Analysis

The overall process of MALDI is shown in Scheme 3.1



Scheme 3.1. Process of MALDI.