SHIMADZU

# Application Notes

# No. AD-0040

**LCMS-8030**

# LC/MS/MS Method for Quantitative Analysis of N-Acylhomoserine Lactones from Sludge Samples

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## Introduction:

Acyl-homoserine lactones (AHLs) are produced by several types of Gram-negative bacteria and act as intercellular signals for bacteria's cell to cell communication. Bacteria regulate gene expression in response to their size population by sensing the level of AHLs that they produce and release to the environment. Here, an analytical method using Shimadzu Nexera UHPLC coupled with LCMS-8030 tandem mass spectrometer for quantitative analysis of three N-Acylhomoserine lactone signal molecules (3-oxo-C6-HSL, 3-oxo-C8-HSL and 3-oxo-C12-HSL) extracted from bio-activated sludge is presented. The LC/MS/MS method employs fast LC separation, fully automatic MRM optimization and two MRM transitions for quantification and confirmation. The result showed that the detection limit of the method was able to reach down to the level of 0.1 ng/ml.

## Method:

Automatic MRM optimization procedure was used to determine the MRM transitions of the standards on LCMS 8030. A pair of MRM transition was chosen for each of the compounds. The one with the higher intensity was used for quantitative analysis whereas the other was for confirmation (Table 1). A fast LC method was established using a Shimpack VP-ODS column (2.1mm ID x 150 mm L, 5 μm); mobile phase: 0.1 % formic acid in water (A) and methanol (B) and a binary gradient elution program (0 min 5%B → 1.00 min 5%B → 1.5 min 50%B → 5.5 min 95%B → 6.5 min 95%B → 6.51 min 5%B → 8.5 min STOP) with a total flow rate of 0.35 ml/min

| Compound Name | MRM Transition | CE (V) | Dwell Time (msec) |
|---|---|---|---|
| 3-oxo-C6-HSL | 214.2 → 102.1 (*) | -10 | 50 |
| | 214.2 → 71.1 (#) | -20 | 30 |
| 3-oxo-C8-HSL | 242.2 → 102.2 (*) | -10 | 50 |
| | 242.2 → 71.3 (#) | -25 | 30 |
| 3-oxo-C12-HSL | 298.3 → 102.1 (*) | -15 | 50 |
| | 298.3 → 71.2 (#) | -25 | 30 |
| Note: * - MRM transition used for quantitation<br># - MRM transition used for confirmation | | | |

**Table 1: MRM transitions used for N-Acylhomoserine lactones analysis**

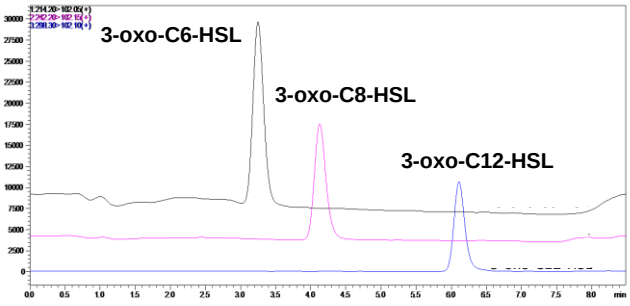
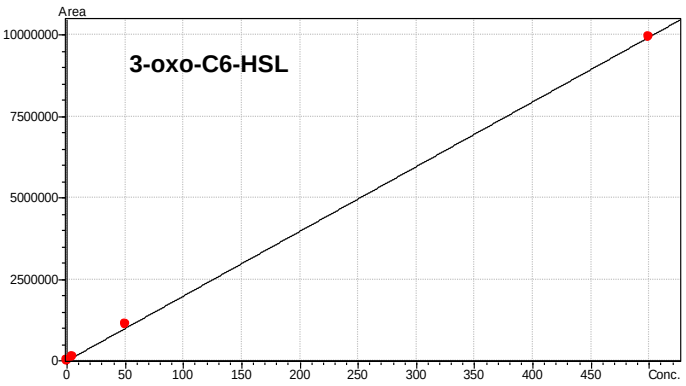



**Fig.1: MRM chromatogram of N-Acylhomoserine lactones mixture at 5ng/ml**

A series of mixed standard samples containing the 3 standards was prepared from stock solutions. The concentration of each standard in the mixed samples was 0.1, 0.5, 5.0, 50.0 and 500.0 ng/ml. Each mixed standard was injected twice, and the result was plotted in duplicates to establish the calibration curve. (Figure 2a-c).




**Concentration : 0.1, 0.5, 5.0, 50 and 500 ng/ml**

**Figure 2a: Calibration curve for 3-oxo-C6-HSL**

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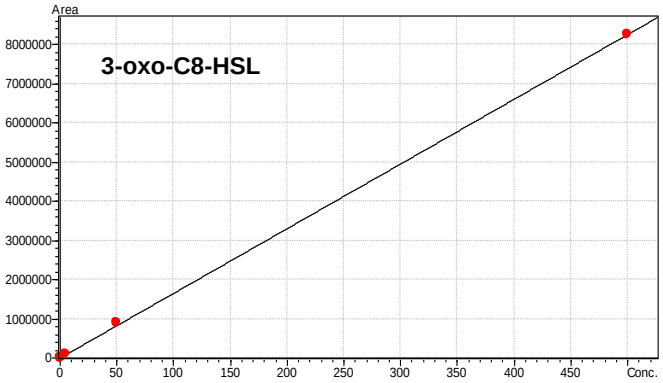
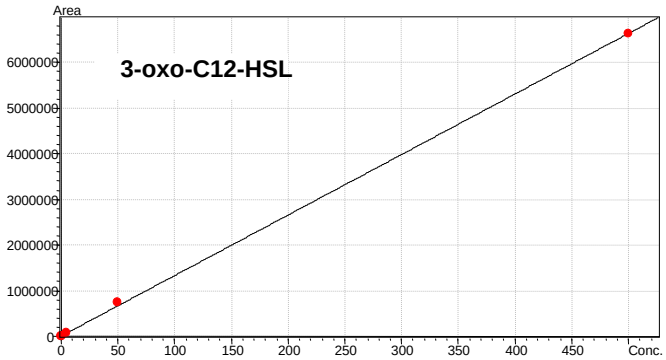


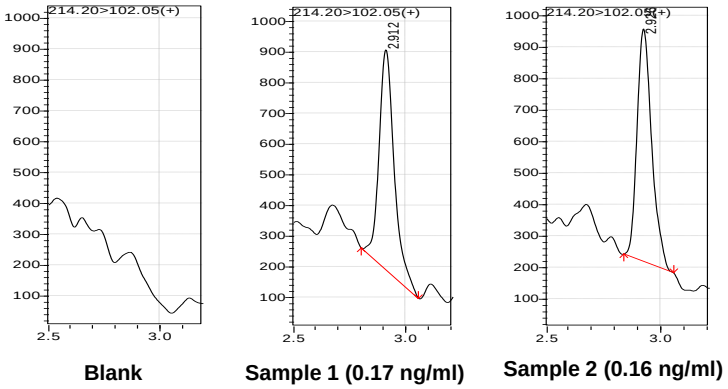

**Concentration : 0.1, 0.5, 5.0, 50 and 500 ng/ml**

**Figure 2b: Calibration curve for 3-oxo-C8-HSL**



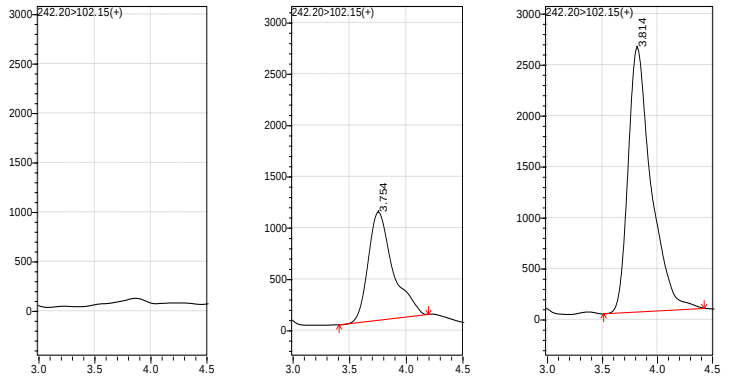

**Concentration : 0.1, 0.5, 5.0, 50 and 500 ng/ml**

**Figure 2c: Calibration curve for 3-oxo-C12-HSL**



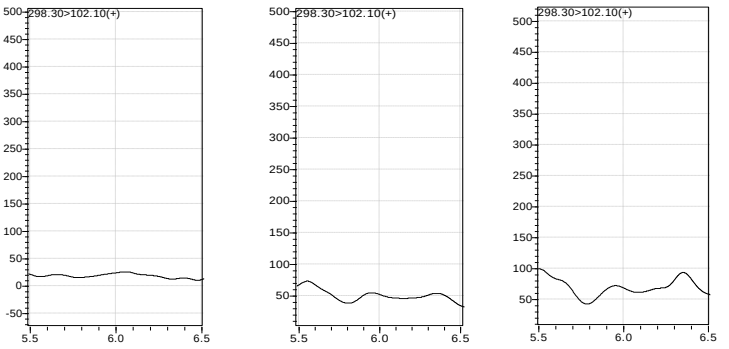

**Blank** **Sample 1 (0.17 ng/ml)** **Sample 2 (0.16 ng/ml)**

**Figure 3a: Result of 3-oxo-C6-HSL**




**Blank** **Sample 1 (0.97 ng/ml)** **Sample 2 (2.12 ng/ml)**

**Figure 3b: Result of 3-oxo-C8-HSL**




**Blank** **Sample 1 (No peak detected)** **Sample 2 (No peak detected)**

**Figure 3c: Result of 3-oxo-C12-HSL**

## Result & Discussion:

Two bio-activated sludge samples (sample 1 and 2) were extracted by liquid-liquid extraction method using dichloromethane. Each sample was evaporated to dryness and reconstituted with 50µL of ethyl acetate acidified with 0.1% acetic acid followed by injecting 20µL of the sample into LCMS-8030 for quantitative analysis.

Figure 3a-c showed the MRM peaks and quantitative results of three N-acylhomoserine lactones in bio-activated sludge samples. The amount of N-acylhomoserine lactones present in sample 1 and 2 were 0.17 ng/ml and 0.16 ng/ml for 3-oxo-C6-HSL (Figure 3a); 0.97 ng/ml and 2.12 ng/ml for 3-oxo-C8-HSL (Figure 3b) and no detection for 3-oxo-C12-HSL (Figure 3c)

## Summary:

A highly sensitive LC/MS/MS method for quantitative analysis of three N-acylhomoserine lactones was established on LCMS-8030 using standard samples in neat solution. Limit of detection (LOD) of the standards is at 0.1 ng/ml.

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