DETECTION OF ENDOTOXINS IN THE ENVIRONMENT BY PYROLYSIS-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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Abstract - The capacity of pyrolysis-gas chromatography-mass spectrometry (Py-GCMS) for analysis of endotoxins is demonstrated in this study. Results obtained for the analysis of endotoxins show that Evolved Gas Analysis can be useful in categorising how endotoxins react to heat, whereas the Single Shot mode can be used to quantitatively correlate peaks observed in the thermogram, and the Multishot Heart Cut method can be used to identify biomarkers of bacteria. The results obtained show that Py-GCMS is a useful technique for the direct analysis of endotoxins.

Keywords - Detection, Endotoxins, Environment, Pyrolysis-Gas Chromatography-Mass Spectrometry.

I. INTRODUCTION

In this study, the pyrolyser is coupled with a gas chromatography-mass spectrometer (GCMS) for method development. The GCMS has the capability of performing unknown identification, but the capacity is limited by the volatility of the sample matrix. Typically, analysis of endotoxins will not be possible for GCMS analysis without elaborate preparatory work. However, the pyrolyser can thermally breakdown the compounds into smaller volatile components. Its use has been demonstrated for analysis of other biopolymers, for example the naturally-formed cellulose, and artificially-made materials.[1] The potential of such instrumental combination for endotoxin analysis has been shown in differentiating Gram-positive from Gram-negative bacteria.[2] Biomarkers of Gram-negative bacteria in Py-GCMS are generally derived from the fatty acids in the lipid A structure; this includes aldehydes and methylalkylketones (from the 3-OH fatty acids bound to the glucosamine backbone), as well as hydrocarbons, fatty acids and nitriles (from secondary acyl chains). The Gram-positive bacteria are marked by the presence of 2-picolinamide.[3] Under heated condition, tetramethylammonium hydroxide (TMAH) functions as a methylating agent, as illustrated in direct analysis in real time-mass spectrometry (DART-MS) analysis.[4] This technique of *in situ* thermally assisted hydrolysis and methylation (THM) using TMAH is also commonly seen in combination with Py-GCMS analyses (THM-Py-GCMS). [5-6] The methyl group replaces labile protons in amine-, hydroxyl-, and carboxyl-groups; this enhances the volatility of the compound for GC-based analysis, and in the process, prevents sample degradation.[7] While there have been applications of Py-GCMS in differentiating bacteria types, the use of this instrument for bacterial detection in environmental samples has not been explored. The use of THM-Py-GCMS can also be examined to evaluate its advantage over using Py-GCMS. This technique potentially offers the ease of sample preparation, and the specificity of a chemical detection technique that does not require the use of chemical standards or biological hosts.

II. DETAILS EXPERIMENTAL

2.1. Materials and Procedures

Using optimised parameters of coupled pyrolyser (Frontier Lab) with GCMS (Shimadzu Corp), endotoxin standards were analysed in Single Shot (SS), Multishot Heart Cut (HC) and Evolved Gas Analyses (EGA). Analysis of lipopolysaccharide standard of *Pseudomonas aeruginosa* 10 (PA10) purchased from Sigma Aldrich was performed. In EGA mode, the pyrolyser heating rate was programmed to be steadily increasing while the GCMS was set to be isothermal with no separating column. The resultant data are thermograms, in which peaks represent the retention of compounds as a function of temperature. An EGA thermogram can be seen to comprise of three major humps (Fig.1). Due to the absence of separation capability in EGA analysis, the components could not be separated into discrete peaks. In other words, each hump or peak in the EGA represents a collection of compounds that elute around the same temperature range. Nevertheless, the thermogram can be useful in categorising how endotoxins react to heat, under inert atmosphere.

The setting in the SS mode is the opposite in EGA; the pyrolyser was set to “isothermal” while the GCMS heating rate was programmed to be steadily increasing, this time using a separating column. In the resultant pyrogram, thermally decomposed products (aka pyrolysate) of endotoxins at 550 ^{o}C (temperature of pyrolyser) were separated by the GC column with high efficiency. As such, the peaks assume their standard retention times based on the affinities towards the stationary phase. Many peaks can be observed, and their identities can be indexed against commercial database under GC-EI-MS analysis. The pyrogram reveals two major peaks: dodecanol and

tetradodecanol (Fig.2). By using the intensities of these two peaks as reference, the other peaks can be quantitatively correlated to these major peaks observed in the EGA thermogram.

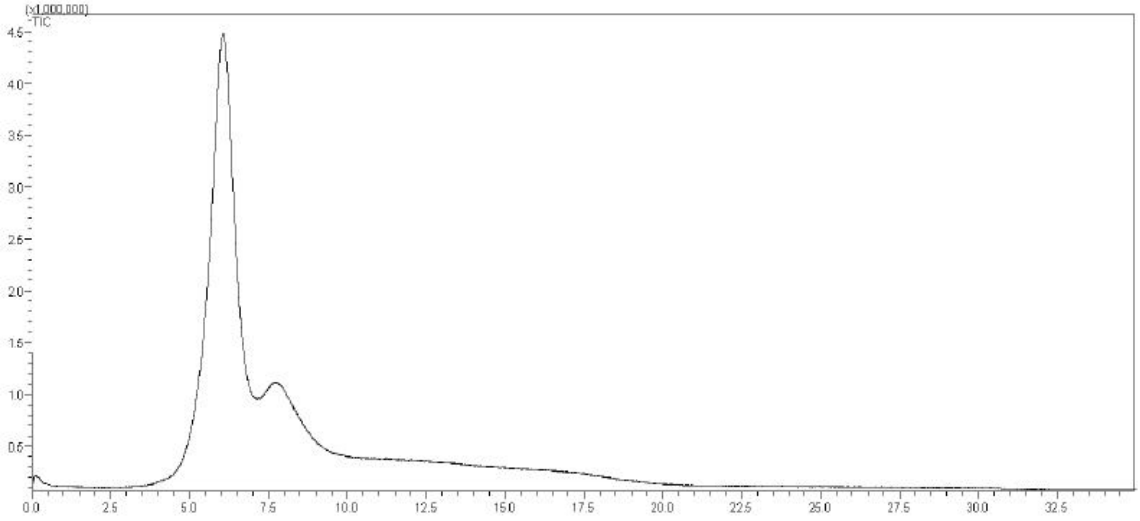


Figure 1 A thermogram for the Evolved Gas Analysis (EGA) of Pseudomonas aeruginosa 10 in Py-GCMS. Three humps depict the thermal decomposition products of endotoxins under inert atmosphere. While the x-axis represents time (in min), it also represents pyrolyser furnace temperature due to the regular heating rate.

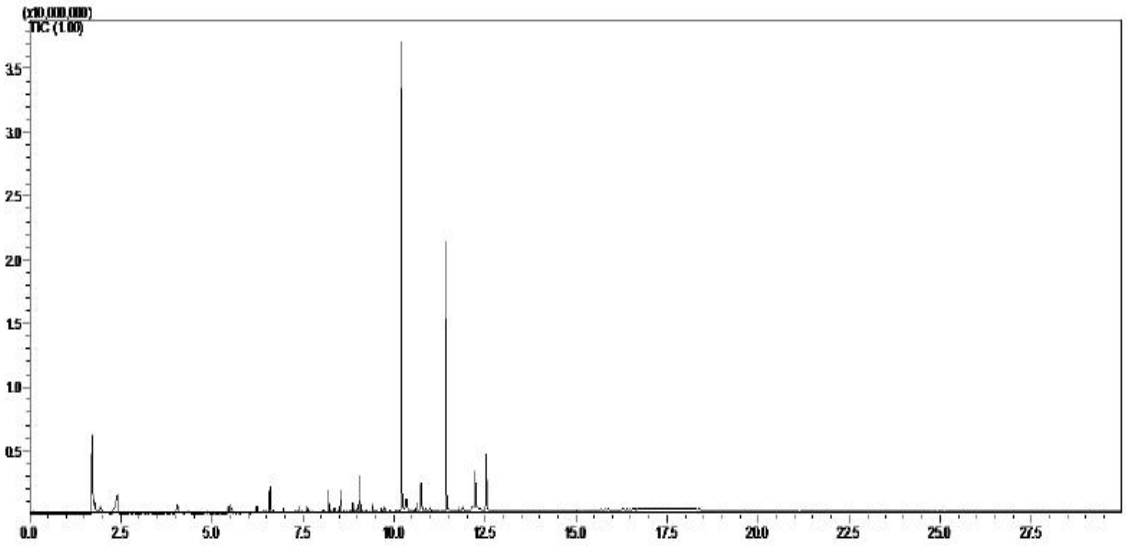


Figure 2 The pyrogram for Single Shot (SS) analysis of Pseudomonas aeruginosa 10 in Py-GCMS. Like any typical GCMS chromatogram, peaks obtained can be searched and matched against database entries. The two major peaks are identified to be dodecanol and tetradodecanol, potentially derived from the lipid A structure.

For confirmation of this correlation, a multishot analysis combining EGA and SS (aka Heart Cut, HC) was performed. According to the retention temperature of the humps in the EGA thermogram, zones were identified for thorough separation in GCMS. Given the three humps in the PA10 thermogram, three respective pyrograms were obtained (Fig. 3). Dodecanol and tetradodecanol were indeed found in the zone A pyrogram. According to a prior study, such hydrocarbons are biomarkers of Gram-negative bacteria.[3]

Upon further investigation, these compounds were found to match those analysed using the lipid A extract from PA10 alone. In order words, it was established that the Py-GCMS is a useful instrument in the direct analysis of lipid A components in bacterial LPS standards.

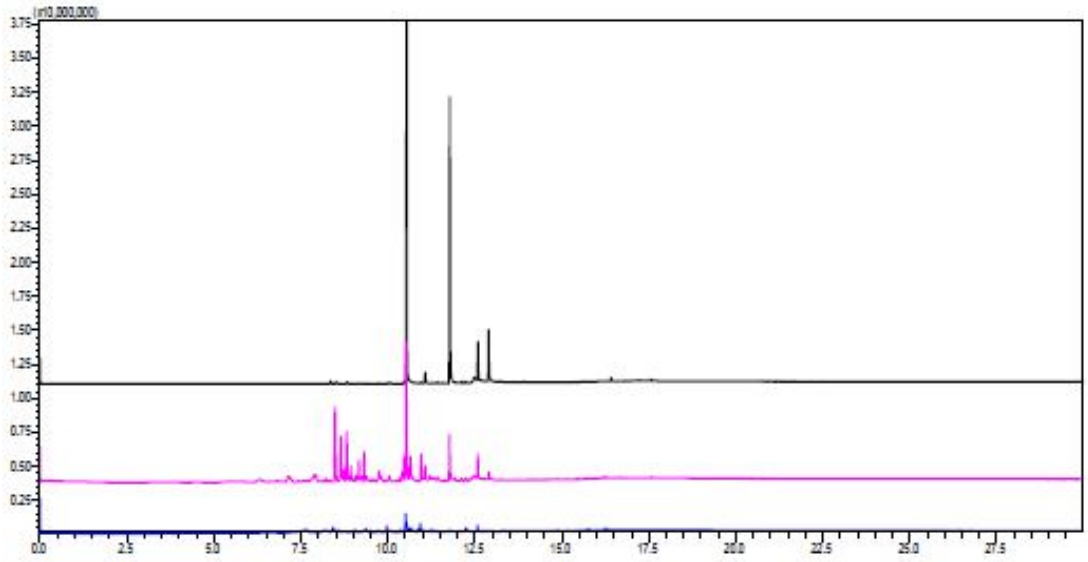


Figure 3 The multishot Heart Cut (HC) analyses of Pseudomonas aeruginosa 10 in Py-GCMS. The pyrograms were obtained according to the three humps in the EGA thermogram (Fig.1). Results are consistent with the SS analysis, in which the major components derived from lipid A: dodecanol and tetradodecanol are found in (A).

CONCLUSIONS

The capacity of Py-GCMS for lipid A analysis in endotoxin standard has been illustrated. Analysis were performed by using methods based on Single Shot, Multishot Heart Cut and Evolved Gas Analyses. Results obtained for the analysis of lipopolysaccharide standard of *Pseudomonas aeruginosa* 10 show that Evolved Gas Analysis can be useful in categorising how endotoxins react to heat, whereas the Single Shot mode can be used to quantitatively correlate peaks observed in the thermogram, and the Multishot Heart Cut method can be used to identify biomarkers of bacteria. With the continuous and progressive improvements in analytical techniques and capabilities, researchers will be able to not only identify precisely, but also to quantify accurately the amount of endotoxins in various sample matrices with ease.

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