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EXACT MASS LC/MS USED TO DETERMINE THE ELEMENTAL COMPOSITIONS OF A FAMILY OF BROMO-BENZYL ALCOHOL SULPHATES FROM *POLYSIPHONIA LANOSA* AND *GIGARTINA STELLATA*

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INTRODUCTION

The marine environment continues to be a source for natural products discovery. The activities of natural marine products can have properties of pharmacological interest, for example, cytotoxicity against tumoral cultured cells, antimicrobial activity and antiviral activity. In addition, anti-feedent activity and insecticidal activity of ecological or agronomical significance has been observed.

Algae are known to produce a wide range of compounds[1] that feature novel structures and often prove to be active in biological systems. However, such species found around the British coasts have been largely ignored. During this study, several algae species were investigated, producing two of particular interest. The first is *Polysiphonia lanosa*, a filamentous, epiphytic red algae collected from Broughty Ferry on the estuary of the River Tay in Scotland, found growing on its host *Ascophyllum nodosum*. The second is *Gigartina stellata,* also a red algae, collected from Arbroath on the east coast of Scotland.

After collection, the samples underwent a series of solvent extraction and partitioning steps to yield hexane, methanol, dichloromethane and water-soluble fractions[2].

Orthogonal acceleration time-of-flight mass spectrometry (oa-TOF-MS), combined with HPLC, proved to be a powerful analytical tool because of its acquisition speed, available mass range, and ion collection efficiency. Within many laboratories the high-duty cycle of TOF is used for qualitative studies, generating full spectra at high mass accuracy (<5 ppm) and providing an extra degree of information that aids in the interpretation of data. Here, fractions were analyzed in 1 mg portions by exact mass LC/MS. The crude extracts generated an overwhelming number of compounds; further work was targeted towards the halogenated compounds found in the water fractions alone. Examples of compounds containing bromine, chlorine and sulphur will be presented.

Investigation focused on the qualitative profiling of *Polysiphonia lanosa* and *Gigartina stellata*. The high sensitivity of HPLC coupled with oa-TOF-MS revealed a complex family of previously unidentified bromo-benzyl alcohol sulphates. By combining the distinctive isotope patterns produced by the bromine, chlorine, and sulphur substituents with exact mass measurements, elemental compositions were conclusively identified. This provided a more complete qualitative profile of the components present in the algae that were responsible for the biological activity observed. From the MS data it was possible to identify 2,3-dibromobenzyl alcohol 4,5-disulphate in the water-soluble fraction of *P.lanosa* and *G.stellata*.

In a previous study, the di-potassium salt was extracted in great quantities from *P.lanosa*[3]. This information, along with the specific exact mass data and the distinctive isotope patterns displayed in the mass spectra, allowed the proposed identification of a family of substituted and sulphated benzyl alcohols produced by the studied organisms.

EXPERIMENTAL

The data was acquired using the Waters® Micromass® LCT Premier™, a new benchtop oa-TOF-MS system. The performance characteristics of TOF technology follow. The resolution capability of TOF technology is shown in Figure 3 for leucine enkephalin, which was used as the reference mass for acquired data.

In Figures 1 to 13, examples of the chromatography and acquired exact mass from the analysis of water fractions from the extracts of *G.stellata* and *P.lanosa* are illustrated. Results were obtained using the extraction procedure, chromatographic conditions, and MS conditions presented.

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Waters Micromass LCT Premier™ performance characteristics

- Elevated mass spectral resolution (W mode >10000 FWHM; V mode > 5000 FWHM).
- Exact mass measurements (<5 ppm RMS);
- Elemental composition determination of target analytes;
- Confidence in confirming target analytes as a means to identify unknowns (using elemental composition calculator);
- Increased selectivity (nominal mass matrix interferences are removed using exact mass chromatograms);
- High sensitivity (efficient "non-scanning" instrument);
- Low-level analyte detection;
- Full-spectrum acquisition;
- Dynamic range (four orders of magnitude).

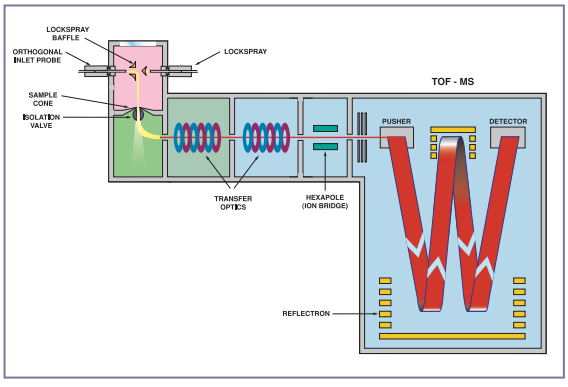


Figure 1. Schematic of oa-TOF.

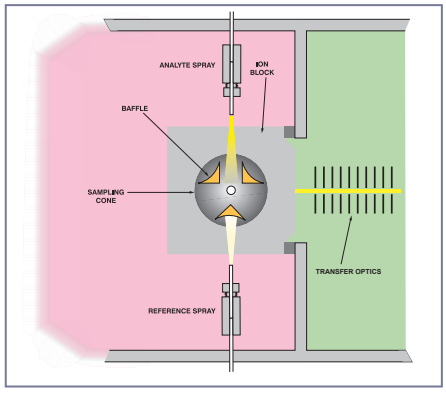


Figure 2. Schematic of LockSpray.

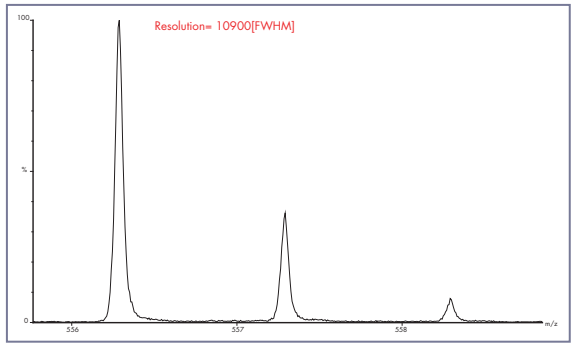


Figure 3: oa-TOF-MS resolution illustration.

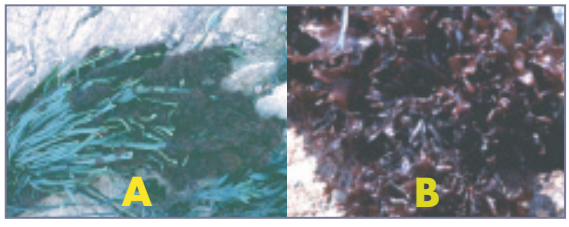


Figure 4: Polysiphonia. lanosa on Ascophyllum nodosum (A) and Gigartina stellata (B).

HPLC Conditions

Column: Waters Symmetry® 5 µm C_{18}, 250 X 4.6

Mobile phase: 0.01 M ammonium formate (pH 7)(solvent A) methanol (solvent B)

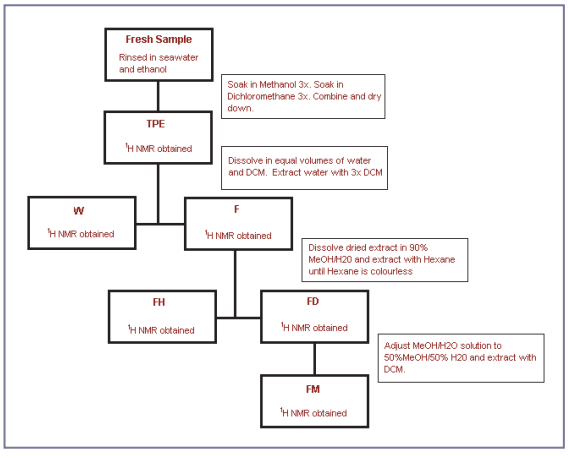
Gradient:
- 0 min: 95% A 5% B
- 30 min: 10% A 95% B
- 32 min: 95% A 5% B
- 35 min: 95% A 5% B

Flow rate: 1mL/min (4:1 split to MS)

MS Conditions

MS: oa-TOF Waters Micromass LCT Premier™

Capillary Voltage:	3000 V
Ionisation mode:	Negative electrospray
Resolution:	5500 (V mode)
Reference Lockmass:	Leucine Enkephalin $[M-H]^-$= 554.2615
Reference frequency:	10seconds



Extraction procedure

RESULTS

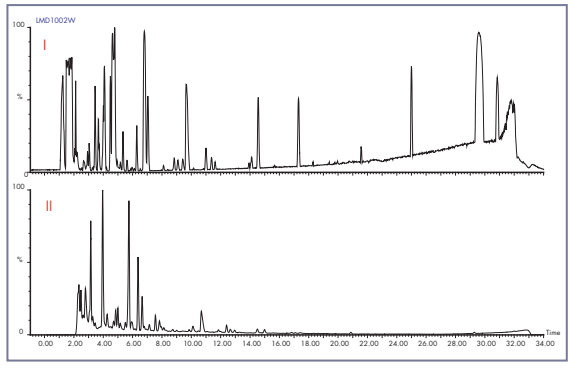


Figure 5. Negative ion mode total ion chromatogram (I) and UV chromatogram λ 254 nm (II) for sample extract LMD1002W from G.stellata.

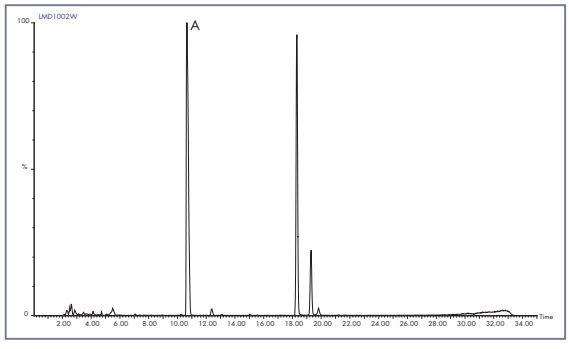


Figure 6. Negative ion mode m/z 454 extracted mass chromatogram for compound A present in the LMD1002W extract from G.stellata.

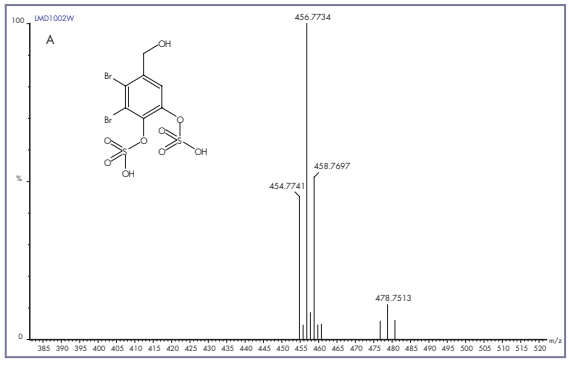


Figure 7. Negative ion mode exact mass spectrum at retention time 10.64 mins for the major di-brominated compound (A) found to be present in extract LMD1002W from G.stellata.

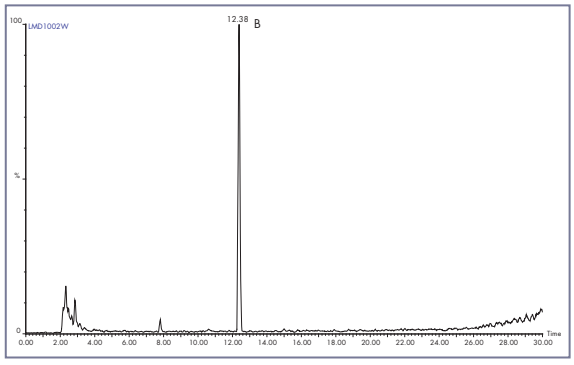


Figure 8. Negative ion mode m/z 532 extracted mass chromatogram for compound B present in the LMD1002W extract from G.stellata.

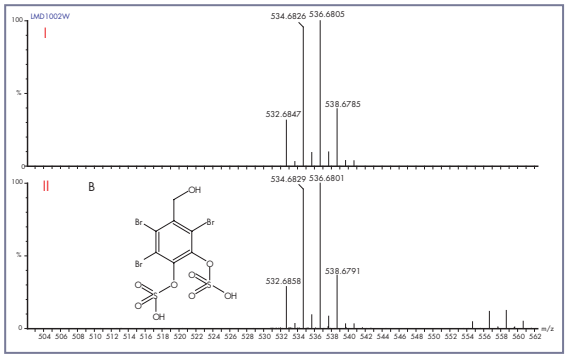


Figure 9. Negative ion mode exact mass spectrum (II) at retention time 12.36 mins for the major tri-brominated compound (B) found to be present in extract LMD1002W from G.stellata. The MassLynx™ theoretical isotope model is illustrated in (I).

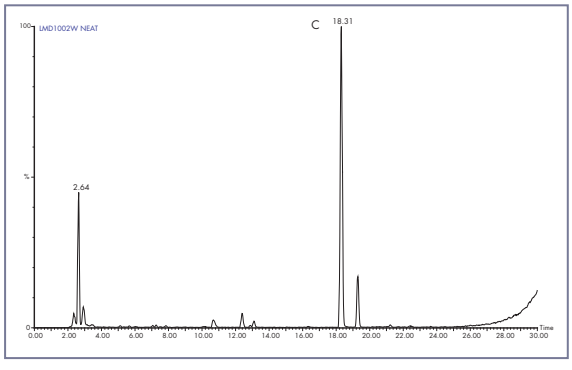


Figure 10. Negative ion mode m/z 452 extracted mass chromatogram for compound C present in the LMD1002W extract from G.stellata.

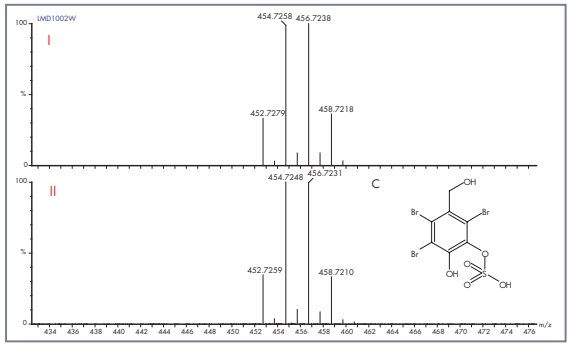


Figure 11. Negative ion mode exact mass spectrum (II) at retention time 18.31 mins for the tri-brominated compound (C) found to be present in extract LMD1002W from G.stellata. The MassLynx theoretical isotope model is illustrated in (I).

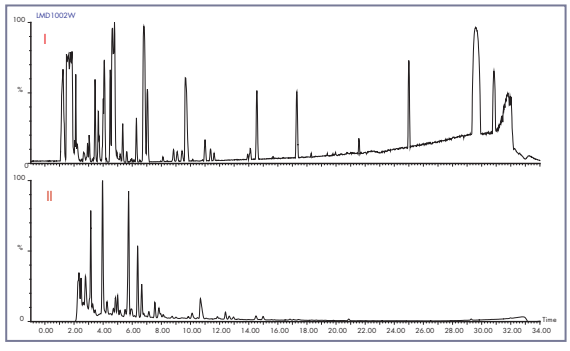


Figure 12. Negative mode total ion chromatogram (I) and extracted mass chromatogram m/z 468 (II) for sample extract LMD302W from P.lanosa, where the major di-bromintated compound A and tri-brominated compound D were determined to be present.

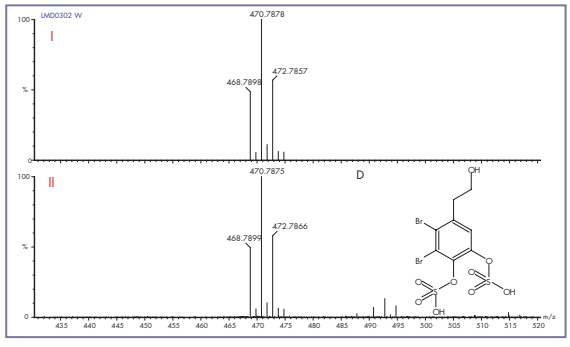


Figure 13. Negative ion mode exact mass spectrum (II) at retention time 12.92 mins for the tri-brominated compound (D) found to be present in extract LMD302W from P.lanosa. The MassLynx theoretical isotope model is illustrated in (I).

DISCUSSION

Using oa-TOF-MS technology and negative mode electrospray ionization in a single analysis of extracts of *P.lanosa* and *G.stellata*, it has been possible to identify numerous major and minor compounds containing bromine, sulfur and chlorine. For this presentation, several examples were chosen to illustrate the power of interfacing HPLC with oa-TOF-MS to produce exact mass measurement. The study was performed using a new Waters Micromass LCT Premier™ benchtop oa-TOF-MS system (see Figure 1) in conjunction with the LockSpray™ source (see Figure 2).

Initial studies combined exact mass measurement with the ability to acquire specific natural isotope ratios, to determine the elemental compositions of compounds extracted from *G.stellata* and *P.lanosa* (see Figure 4). This information, combined with that of a previous study, has enabled the proposal of structures of compounds A, B, C and D[3].

From Figure 5, it can be seen that many compounds of interest were not observed via UV detection. The distinctive isotope pattern of a di-brominated compound is seen in Figure 7, for which compound A is illustrated with its related extracted mass chromatogram in Figure 6. The observed isotope pattern indicated a di-brominated species. The elemental composition was determined to be $C_7H_5Br_2O_9S_2$, and the observed isotope pattern was a perfect match for 2,3-dibromobenzyl alcohol 4,5-disulphate. In addition all three major isotopes were observed within 5 ppm of their calculated m/z.

This compound formed one of the most abundant species determined to be present in the *G.stellata*. At retention time 12.36 minutes for the extract of *G.stellata*, another distinctive bromine isotope pattern was seen, in this case indicative of a tri-brominated compound. The elemental composition $C_7H_4Br_3O_9S_2$, was determined within 5 ppm mass accuracy. The exact mass spectrum is shown in Figure 9, along with the proposed structure; the corresponding extracted mass chromatogram is shown in Figure 8.

In Figure 7, use of the theoretical isotopic model within MassLynx™ software is also presented, demonstrating an excellent match between the theoretical and measured isotopic abundances. For compound C which elutes at 18.31 minutes (see Figure 10) in the m/z 452 extracted mass chromatogram, a deprotonated elemental composition of $C_7H_4Br_3O_6S$ was determined. The exact mass spectrum is presented in Figure 11, where the MassLynx™ software's theoretical isotope model and the acquired isotope distribution show excellent comparibility. The isotope pattern observed indicates a tri-brominated compound, and from the exact mass measurement only one sulphate substitution is present; the structure of compound C was proposed from this information.

Polysiphonia lanosa also contained numerous brominated species. The major compound identified in *P.lanosa* was 2,3-dibromobenzyl alcohol 4,5-disulphate, as shown in Figure 12 in the total ion chromatogram (compound A). Also illustrated is the m/z 456 extracted mass chromatogram for compound D, which elutes at 12.92 minutes. The exact mass spectrum obtained for compound D is shown in Figure 13 for which the deprotonated elemental composition determined was $C_8H_7Br_2S_2$, along with the MassLynx theoretical isotope model. The isotope distribution obtained indicated a di-brominated compound, the exact mass data obtained indicated a m/z difference relating to CH_2, between compound A and D, hence the structure of compound D was proposed.

CONCLUSION

- Oa-TOF technology enables large numbers of major and minor unknown compounds to be identified in one analysis.
- Exact mass measurement within 5 ppm is routinely achieved.
- Excellent correlation between theoretical and measured isotope distribution has been achieved with exact mass measurement within 5 ppm on all major isotopes determined.
- The high sensitivity of HPLC coupled to an oa-TOF-MS has revealed a complex group of previously undetected minor compounds containing a combination of bromine, sulphur and chlorine.
- The combination of exact mass measurement and correct isotope distribution determination has allowed the structures presented to be proposed.
- Exact mass measurements have been used to identify the elemental compositions of major and minor compounds in an attempt to obtain a more complete profile of *P.lanosa* and *G.stellata*.

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[3] Hodgkin J.H., Can. J. Chem., **1966**, 44, 74-78

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