A comparison of HPLC MS methods for GDGT analysis; should we make the switch?

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Aim

To explore potential improvements of GDGT analysis by reverse phase (RP) ultra high performance liquid chromatography mass spectrometry UHPLC-MS, compared to existing normal and reverse phase methods.

Introduction

Although GDGT analysis using normal phase atmospheric pressure chemical ionization (NP-APCI-HPLC) MS is a well-established method (Hopmans *et al*., 2000), our experience has been one of recurring pressure fluctuations, caused by the behaviour of hexane under high pressure in a serial dual piston HPLC pumping system (Fig. 1). This problem is not uncommon. In addition, the NP method is not amenable to UPLC.

Therefore, we started to explore the possibility of setting up reverse phase HPLC, following the recent work of Zhu *et al*., (2013) and reverse phase methods used in purification schemes (Ingalls *et al*., 2006; Birkholz *et al.*, 2013).

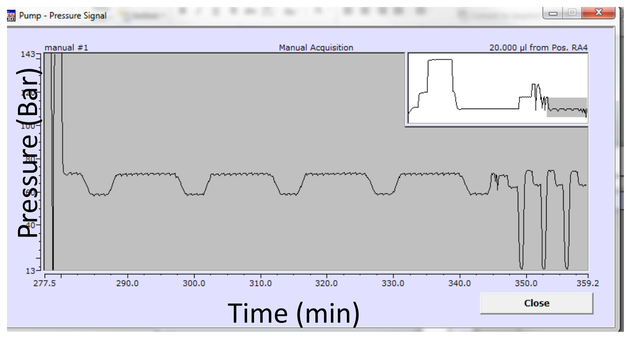

Figure 1. An example of the erratic pump pressure when pumping Hexane: IPA (99:1%)

Methods

Core GDGTs were extracted from freeze dried sediment using either ultrasonic extraction or microwave extraction. Samples were passed over a small silica column, dried using a vacuum concentrator, dissolved in MeOH and filtered through a 0.45µm syringe filter. Measurement was performed using the instrument described in Fig. 1, scanning over *m/z* 1017-1024; 1031-1038; 1045-1052; and 1290-1304. NP chromatography was set up and performed as described by Hopmans et al., 2000. RP chromatography was performed on a reverse phase UHPLC column from Phenomenex. Mobile phase A was MeOH and mobile phase B Isopropyl alcohol, both with formic acid. The total run time was 38 minutes including column re-equilibration.

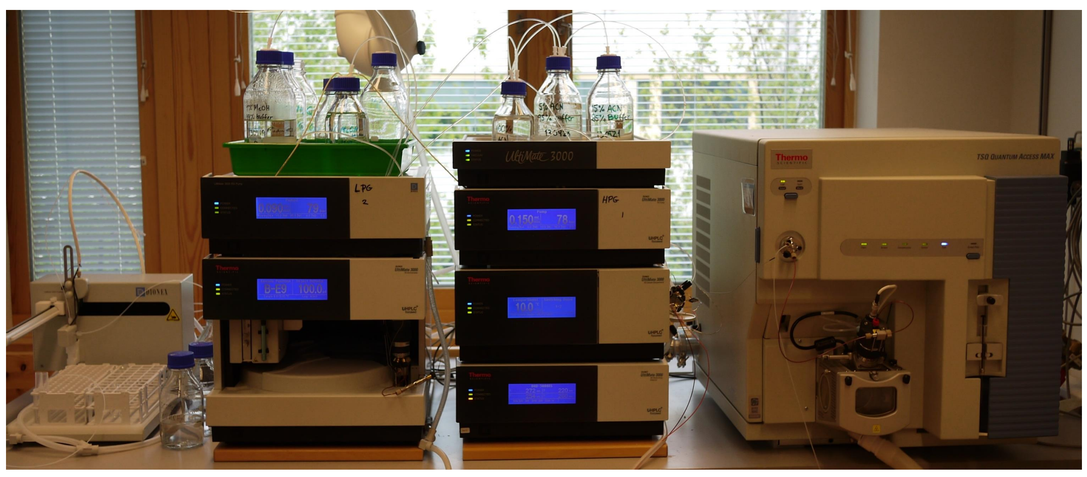

Figure 2. The HPLC MS/MS system used; a Thermo-Dionex Ultimate 3000RS Quaternary Rapid Separation Pump LPG-3400RS (left), auto sampler and column compartment, linked to a Thermo TSQ quantum access max triple stage quadrupole.

Conclusions

Compared to the established NP HPLC method, this RP UHPLC method:

- gives a very stable back pressure and is set up easily
- gives an improved separation of the branched GDGTs, within 15 minutes.
- gives a similar separation and ret. time of the isoprenoid GDGTs
- gives a (different) separation of structural isomers, allowing to further explore GDGT sources and potential novel proxies.
- APCI appears to work better than ESI ionization

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Results and Discussion

Ionization: ESI vs APCI

The effect of ionization source was investigated by running the method using APCI and ESI (Fig. 3). Although ESI gave a higher response, it also gave a high background and we observed suppression of some compounds e.g. m/z = 1296. APCI was selected for further use in the method.

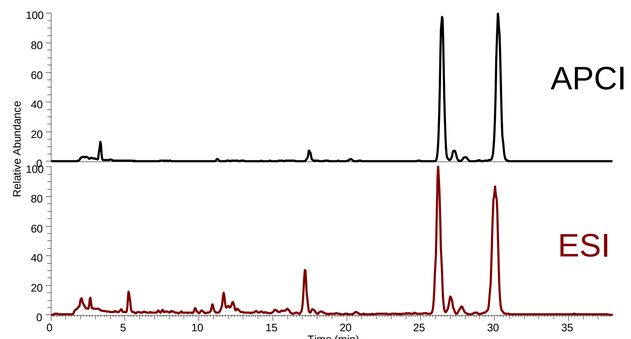

Figure 3. Comparison of ESI with APCI ionization. Base Peak chromatograms of a Black Sea sediment extract are shown.

Chromatography and separation of GDGTs

Figure 4a. shows the separation of isoGDGTs using the RP method. Note the peaks of m/z 1300, 1298 1296 and 1292 that elute around 15 min, these are suspected to be isomers of the main peaks eluting between 25-30 min.

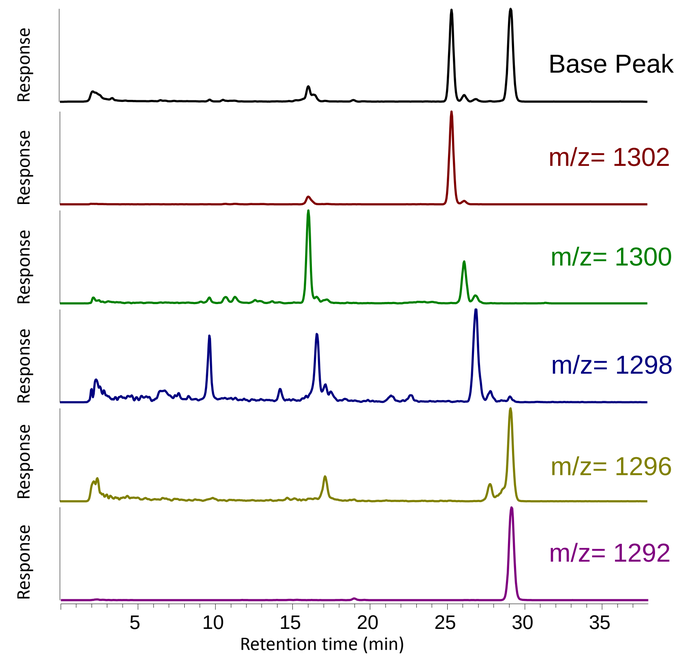

Figure 4a. BP and extracted ion chromatograms of isoprenoid GDGTs from a Black Sea sediment sample, using the RP method.

Fig 4b. shows the separation of brGDGTs. Note that they elute before the isoGDGTs, in contrast to the NP method.

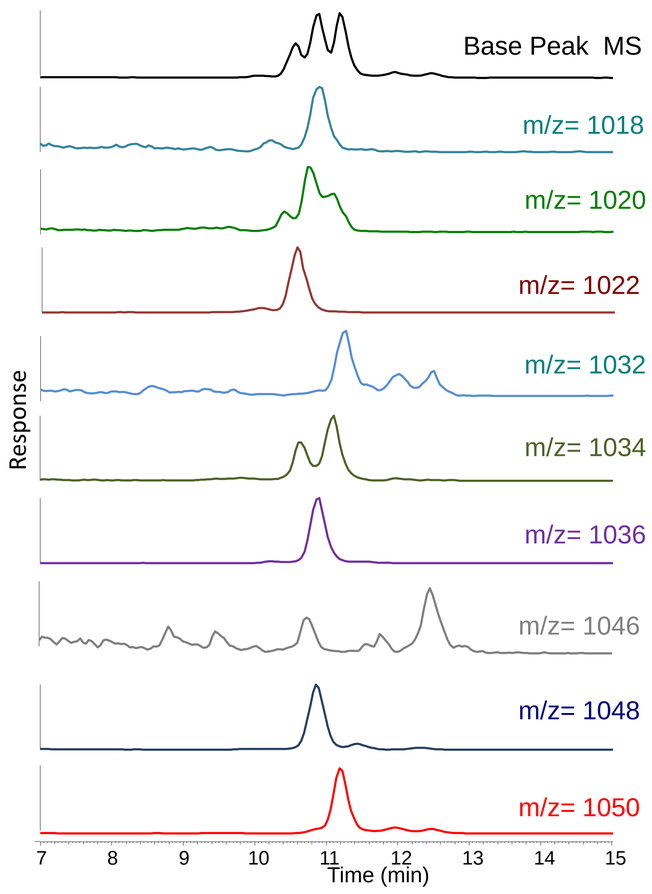

Figure 4b. BP and extracted ion chromatograms of branched GDGTs from a Hässeldala (SE) lake sediment sample

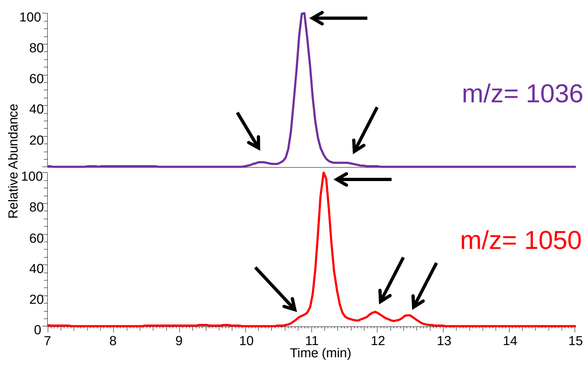

Figure 4c. Expanded view of the 1036 and 1050 extracted ion chromatograms and potential isomers

Fig. 4c. A series of peaks with the same m/z (e.g. 1036 and 1050) suggest that various structural isomers of the brGDGTs are near-baseline separated. If true, this would allow further exploration of the sources of the brGDGTs and potential environmental controls of their relative abundance.

Future work

- Resolving the regio-isomer of crenarchaeol (1292r) from the main isomer
- Resolving the issue of apparent relative difference in abundance of the 1020 GDGTs, when compared to the NP method

References

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