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Compound-specific radiocarbon analyses of individual fatty acids isolated with high performance liquid chromatography

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Long-chain fatty acids (LCFAs, > 22 C atoms) are primarily biosynthesized by terrestrial higher plants and are therefore typically regarded as biomarkers for terrestrial organic matter, although they are also found in some phytoplankton species. Because of their specificity, their ubiquitous nature, and relatively ease of separation from total organic lipid extracts, they have been prime targets for compound-specific radiocarbon analysis (CSRA) (Eglinton, 1996). This technique has been applied mainly to elucidate questions regarding terrestrial organic carbon cycling (e.g. Matsumoto, 2007), but also for dating purposes (e.g. Ohkouchi, 2003)
Until recently, the isolation protocol (Mollenhauer, 2009) required some chromatographic steps to obtain a semi-pure LCFAs fraction. These LCFAs had to be methylated and separated with preparative gas chromatography (prepGC). The main disadvantage of this technique is the addition of an extraneous methyl group with a different 14C signature. Moreover, a minimum of 50 injections is needed to yield sufficient amounts of the individual FAs for radiocarbon analysis. This increases the danger of further contamination with extraneous carbon.
We present a new method to purify individual LCFAs by the use of semi-preparative high performance liquid chromatography (HPLC). We were successful in separating individual LCFAs without detectable contamination using a reversed phase C18 column and a mobile phase with a mixture of acetonitrile, ethyl acetate and methanol. The radiocarbon content of the separated compounds were measured using a MIni CArbon DAting System (MICADAS) equipped with a gas ion source, that allows the measurement of samples down to 3μg C (Ruff, 2006). With the new LC method the number of injections could be reduced dramatically compared to the prepGC, because the capacity of the LC column is much bigger. A second advantage is that the addition of extraneous C to the compounds could be avoided, as no derivatizion was needed. Third, the presented method can likely be expanded and optimized towards the separation of fatty acids with different C chain lengths or towards the more polar hydroxy-fatty acids.
The new method was tested with fatty acid standards (C21-FA, C26-FA and C28-FA) with different radiocarbon signatures (60 - 100 percent modern). We also separated individual LCFAs from a natural peat and compared the purification and radiocarbon results of the GC and the new LC method. We also tested the effect of varying sample sizes (3 to 30μg C) before and after the isolation procedure with HPLC.

References

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