

New stable isotope enriched plant compounds for use as internal standards



Ries de Visser¹, Arno Hazekamp² & Ton Gorissen¹

¹ IsoLife BV, Bornsesteeg 65, NL-6708 PD Wageningen, info@isolife.nl, www.isolife.nl

² Prisna BV, Gorlaeuslaboratoria, Einsteinlaan 55, NL-2333 CC Leiden, www.prisna.nl

IsoLife BV produces plant components, uniformly (U) labelled with stable isotopes like ¹³C and ¹⁵N. They are obtained from food plants and pharmaceutical herbs cultivated in advanced phytotrons, which are specifically designed for high abundance isotope labelling.

These innovative components are applied as internal standards for MS in medicine, nutrition, clinical chemistry, and biotechnology (metabolomics). One example of a new product is the labelled flavonoid U¹³C-quercetin (Fig. 1).

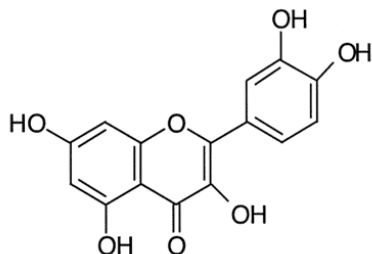


Figure 1. Quercetin

Materials and Methods

Potato (cv. 'Elkana') and tomato (cv. 'Microtom') plants were grown in an Espas labelling chamber (1) on a hydroponics system (2). ¹³CO₂ (99 atom %; Isotec) was the only C source. U¹³C-quercetin was extracted from potato shoots by methanol/ water, acid hydrolysis and CPC fractionation and analyzed by HPLC and MS by Prisna BV. Tomato fruits of different ripening stages were flash-frozen in LN₂.

Results

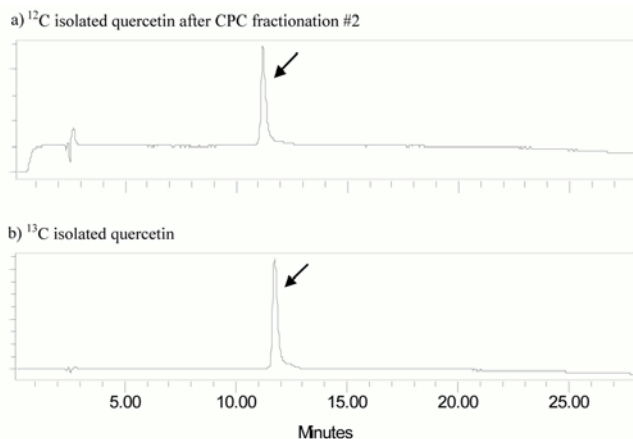


Figure 2.

U¹³C-quercetin has been purified from U¹³C-plants by HPLC and CPC as illustrated in Figure 2 and demonstrated by MS analysis (Figure 3). The latter also shows the uniform ¹³C labelling (> 98.5 atom %; binomial distribution), since molecular mass increased from 303.2 to 318.2 (quercetin has 15 C-atoms).

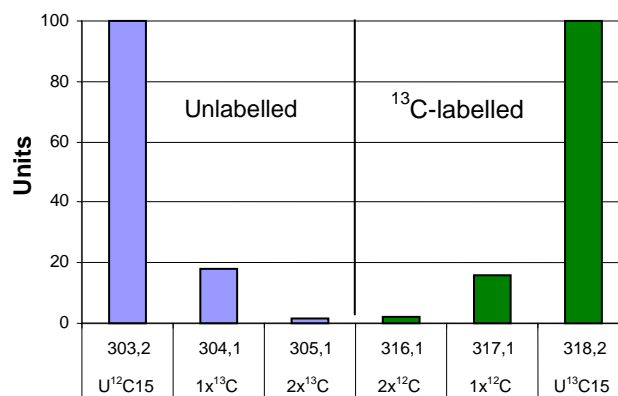


Figure 3. [M+H]⁺ Molecular Ion Mass of Quercetin

Discussion

Two advantages of U¹³C-standards are:

- they facilitate identification (LC-MS) by indicating the number of carbon atoms
- they improve quantification of multiple metabolites in extracts (GC-MS) by reducing variation by a factor six.

Future products involve U¹³C-tomato fruit extracts and software for high-throughput analyses. This technique will allow automated and quantitative analyses of complete 'metabolomes' in nutrition, medicine, plant breeding, and pharmaceuticals (3,4,5).

IsoLife BV provides powerful stable isotope tracers for a range of applications. We create tailor-made solutions for your specific problems.



References

1. Gorissen A et al. 1996. ESPAS - An advanced phytotron for measuring carbon dynamics in a whole plant-soil system. *Plant and Soil* 179: 81-87.
2. De Visser R et al. 1992. In: *Molecular, Biochemical and Physiological Aspects of Plant Respiration*. Eds Lambers H & Van der Plas LHW, pp 493-508. Academic Publishing, The Hague.
3. Hall RD. 2006. *Plant metabolomics: from holistic hope, to hype, to hot topic*. *New Phytologist* 169: 453-468.
4. De Vos RCH et al. 2007. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nature Protocols* 2: 778-791.
5. Verhoeven HA, et al. 2006. Plant metabolomics strategies based upon quadrupole time of flight mass spectrometry (QTOF-MS). In Saito K et al. (Eds.), *Plant Metabolomics* (57) pp. 33-48. Berlin, Springer-Verlag.

