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Supplement of

Quantification of lignin oxidation products as vegetation biomarkers in speleothems and cave drip water

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Supplement to "Quantification of lignin oxidation products as vegetation biomarkers in speleothems"

I. Heidke, D. Scholz, T. Hoffmann

S1 Evaporation effects of different elution solvents for SPE
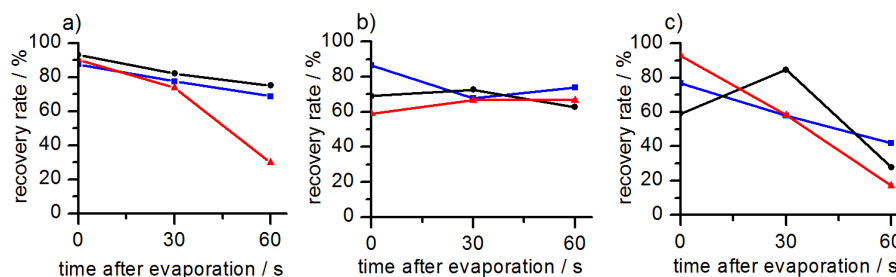

Figure S1. Recovery rates of vanillin after evaporation in a) acetonitrile, b) methanol and c) ethyl acetate at 45 °C (red triangles), 30 °C (black circles) and 25 °C (blue squares). The residue was re-dissolved in H_2O/ACN 9:1 (v/v) and analyzed. At elevated evaporation temperatures, vanillin and other aldehydes evaporated and were lost for analysis. In ethyl acetate, this evaporative loss was more pronounced than in acetonitrile and methanol.
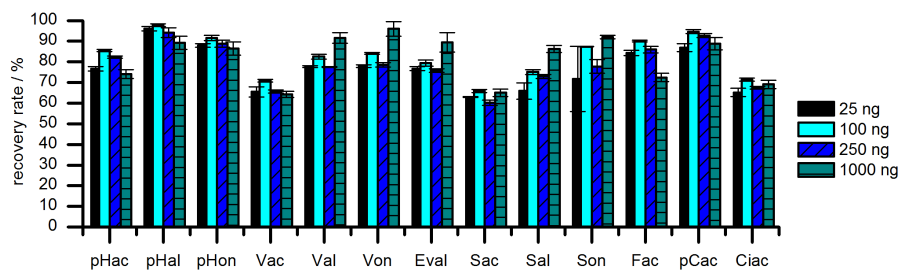
Figure S2. Recovery rates of the solid phase extraction of LOPs at different spiking concentrations. 20 mL of a surrogate sample solution $(2 \; \text{mol} \cdot \text{L}^{-1}$ NaCl in ultrapure water, acidified with HCl to pH 2) were spiked with 25, 100, 250 and 1000 ng of LOP standards.

Figure S3. Linearity test of the SPE method for the extraction of LOPs. 20 mL of a surrogate sample solution $(2 \; \text{mol} \cdot \text{L}^{-1}$ NaCl in ultrapure water, acidified with HCl to pH 2) were spiked with 25, 100, 250 and 1000 ng of LOP standards.
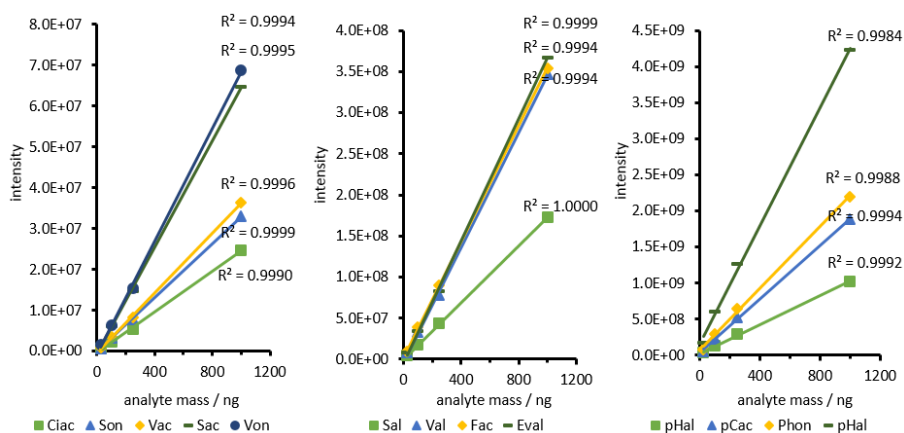
S3   Test of the addition of glucose to prevent overoxidation

Many studies (e.g., Kaiser and Benner, 2012; Louchouarn et al., 2000; Spencer et al., 2010) recommended to add glucose to samples with low organic carbon content to prevent overoxidation of aldehydes. As stalagmite samples do have a low organic carbon content compared to soil or sediment samples, we tested the addition of glucose. The result was that the ratio of Vac/Val did indeed decrease from 0.48 ± 0.11 without glucose to 0.26 ± 0.12 with glucose, because the yield of vanillin increased with the addition of glucose. Nevertheless, the ratio of C/V decreased from 0.46 ± 0.12 to 0.26 ± 0.31 and the ratio of S/V decreased from 0.76 ± 0.19 to 0.41 ± 0.36 (Fig. S4). This means that the addition of glucose did not prevent cinnamyl and syringyl phenols from overoxidation. In contrast, there were more interfering peaks in the chromatograms with glucose (Fig. S5), which made integration difficult and lead to increased uncertainty in quantification. Consequently, no glucose was added in the CuO oxidation step.
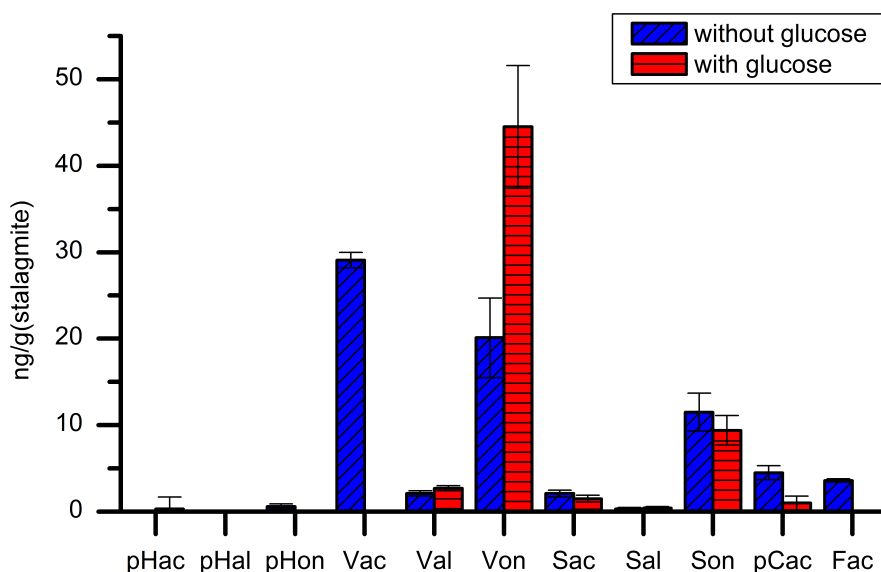

Figure S4. Comparison of LOP concentrations with and without the addition of glucose. Only for Von, there was an increase in the concentration with the addition of glucose. For all other analytes, the method without glucose gave better results.
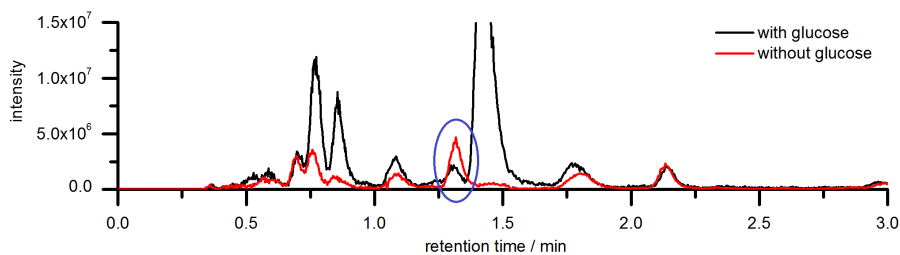
Figure S5. Chromatogram of *m/z* 167.03498 (vanillic acid) with (black line) and without (red line) the addition of glucose. The peak of vanillic acid is circled. It was higher and better separated from neighbouring peaks without the addition of glucose. Similar observations were made for other analytes, too.

S4 Linear regresssion parameters of the external calibration functions and instrumental limits of detection (LOD) and qualibration (LOQ).

Table S1. Linear regresssion parameters of the external calibration functions and instrumental limits of detection (LOD) and qualibration (LOQ).

analyte	R^2	slope	intersept	instrumental LOD / $ng \cdot mL^{-1}$	instrumental LOQ / $ng \cdot mL^{-1}$
pHac	0.9998	6371269	229351	0.39	1.19
pHal	0.9949	24875695	10116687	0.05	0.15
pHon	0.9988	14313979	1679485	0.18	0.55
Vac	0.9998	206028	3287	0.48	1.46
Val	0.9997	630114	39639	0.25	0.75
Von	0.9962	129627	-6652	2.27	6.89
Sac	0.9993	270474	-23398	0.55	1.66
Sal	0.9996	170558	-17658	3.75	11.36
Son	0.9998	122729	3837	7.92	24.00
pCac	0.9978	11971947	2189589	1.08	3.26
Fac	0.9998	2028750	-169451	0.10	0.29
Eval	0.9998	1769186	929	0.24	0.73
Ciac	0.9996	100371	7569	4.44	13.45

S5 Equations used for calculation of concentrations, lignin oxidation parameters and errors bars

The concentration c(analyte) of real samples was calculated by equation (1), with A = mean peak area of three LC-MS analyses of the sample, B = mean peak area of three LC-MS analyses of the blank sample, b = intersect of the Y-axis of the external calibration curve, m = slope of the external calibration curve, f_r = recovery factor of the internal standard ethylvanillin (Eval), see equation (2), V = volume of the final sample solution and m_{sample} = sample mass.

$$c(\text{analyte}) = \frac{A - B - b}{m} \cdot \frac{1}{f_r} \cdot \frac{V}{m_{\text{sample}}} \tag{1}$$

$$f_r = \frac{c(\text{Eval})_{\text{measured}}}{c(\text{Eval})_{\text{spiked}}} \tag{2}$$

The error Δc(analyte) of the concentration c(analyte) was calculated by equation (3).

$$\Delta c(\text{analyte}) = \sqrt{\left(\frac{\partial A}{\partial c}\Delta A\right)^2 + \left(\frac{\partial B}{\partial c}\Delta B\right)^2 + \left(\frac{\partial b}{\partial c}\Delta b\right)^2 + \left(\frac{\partial m}{\partial c}\Delta m\right)^2 + \left(\frac{\partial f_r}{\partial c}\Delta f_r\right)^2 + \left(\frac{\partial m_{\text{sample}}}{\partial c}\Delta m_{\text{sample}}\right)^2} \tag{3}$$

The lignin oxidation parameters were calculated according to equations (4) to (9). Their errors were calculated using the law of propagation of uncertainty (equations not shown).

$$\text{C-group LOPs} = c(\text{p-Cac}) + c(\text{t-Fac}) \tag{4}$$

$$\text{S-group LOPs} = c(\text{Sac}) + c(\text{Sal}) + c(\text{Son}) \tag{5}$$

$$\text{V-group LOPs} = c(\text{Vac}) + c(\text{Val}) + c(\text{Von}) \tag{6}$$

$$\Sigma 8 = \text{C-group LOPs} + \text{S-group LOPs} + \text{V-group LOPs} \tag{7}$$

$$\text{C/V} = \frac{\text{C-group LOPs}}{\text{V-group LOPs}} \tag{8}$$

$$\text{S/V} = \frac{\text{S-group LOPs}}{\text{V-group LOPs}} \tag{9}$$

References

Kaiser, K. and Benner, R.: Characterization of Lignin by Gas Chromatography and Mass Spectrometry Using a Simplified CuO Oxidation Method, Analytical chemistry, 84, 459–464, 2012.

Louchouarn, P., Opsahl, S., and Benner, R.: Isolation and Quantification of Dissolved Lignin from Natural Waters Using Solid-Phase Extraction and GC/MS, Analytical chemistry, 72, 2780–2787, 2000.

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