

# ***Additional File 1***

## ***Corynebacterium glutamicum* as platform for the production of hydroxybenzoic acids**

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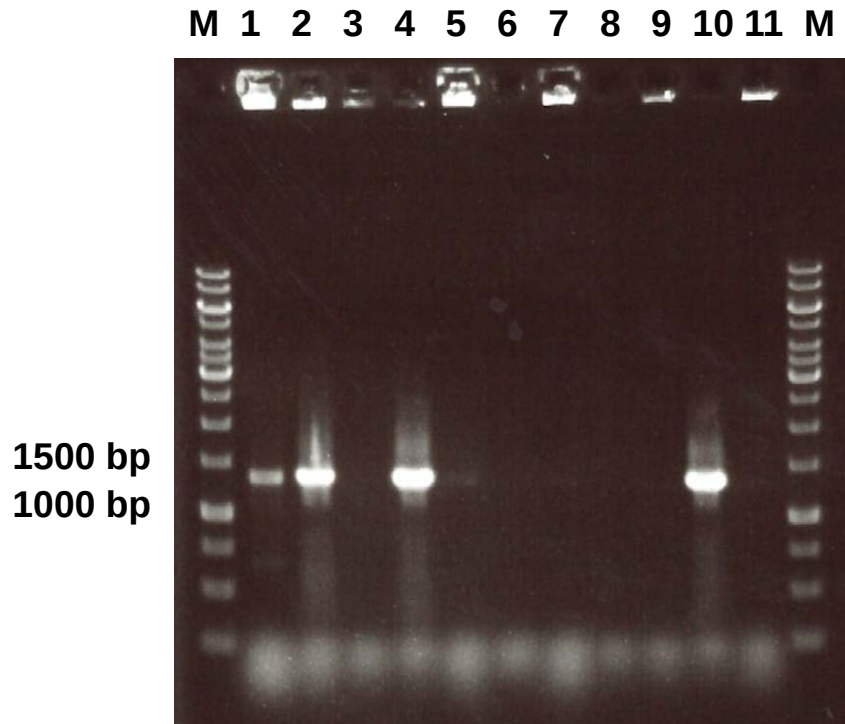
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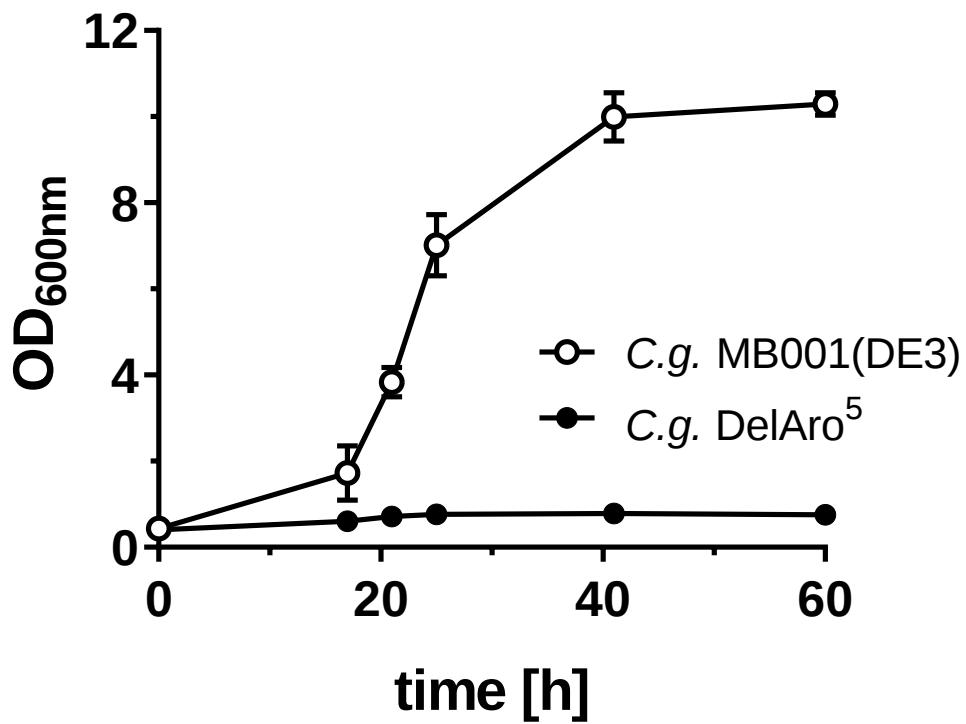
### **This additional file contains:**

- Fig. S1      Confirmation of the successful deletion of the nag-gene cluster by PCR
- Fig. S2      Cultivation of *C. glutamicum* strains with 3-hydroxy benzoate as sole carbon and energy source
- Fig. S3      LC-MS analysis of microbially produced PC
- Fig. S4      LC-MS analysis of microbially produced 3-HB
- Fig. S5      LC-MS analysis of microbially produced 4-HB
- Fig. S6      LC-MS analysis of microbially produced 2-HB
- Fig. S7      Extracted chromatograms for m/z of benzoate and of 2-HB



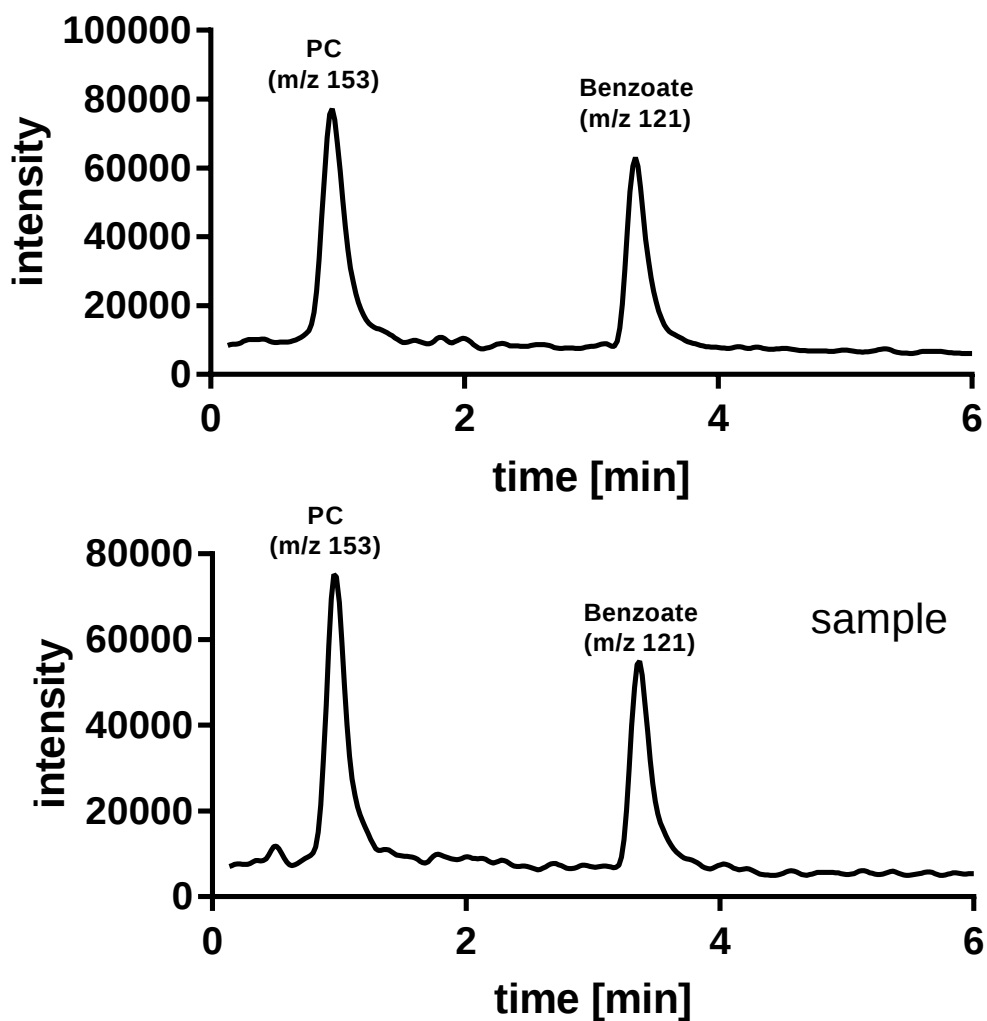
**Additional File 1: Fig. S1. Confirmation of the successful deletion of the *nag*-gene cluster by PCR**

The deletion of cg3349-cg3354 (*nagIKL-nagR-nagT-genH*) in *C. glutamicum* DelAro<sup>4</sup> yielding *C. glutamicum* DelAro<sup>5</sup> was confirmed by colony-PCR and subsequent agarose gel electrophoresis. For this purpose, oligonucleotides *check\_cg3349-54-s* and *check\_cg3349-54-as* were designed bind up- and downstream of the flanking regions targeted during for homologous recombination, respectively. Successful deletion of the five genes corresponded to a PCR product of 1332 bp, whereas no PCR product was expected for the wild-type situation as the resulting PCR product of 7701 bp was too long to be amplified. Clone 4 (lane 4) was selected for subsequent strain engineering towards microbial production of aromatic compounds.



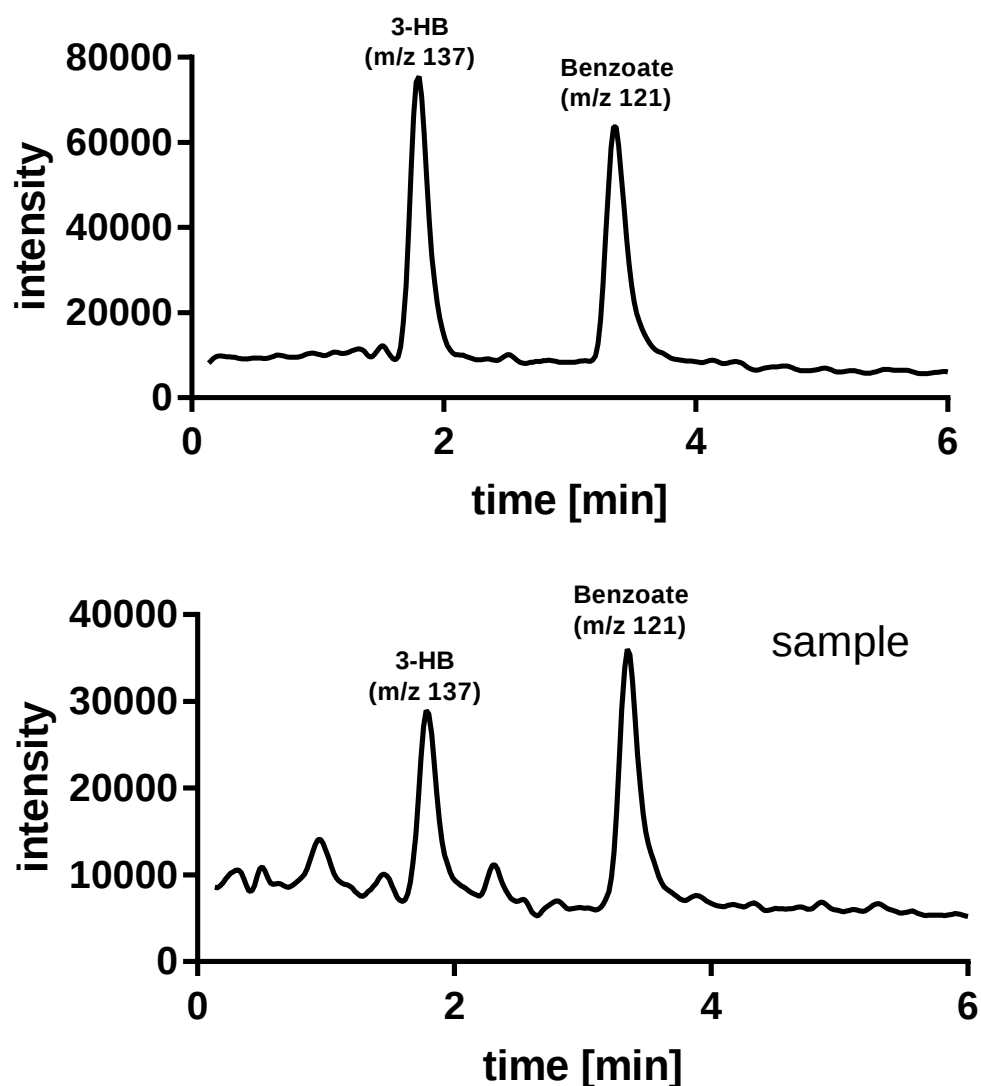
**Additional File 1: Fig. S2. Cultivation of *C. glutamicum* strains with 3-hydroxybenzoate as sole carbon and energy source**

Successful deletion of cg3349-3354 was also confirmed by cultivation of *C. glutamicum* DelAro<sup>5</sup> and of the reference strain *C. glutamicum* MB001(DE3) in defined CGXII medium with 20 mM 3-hydroxy benzoate as sole carbon and energy source.



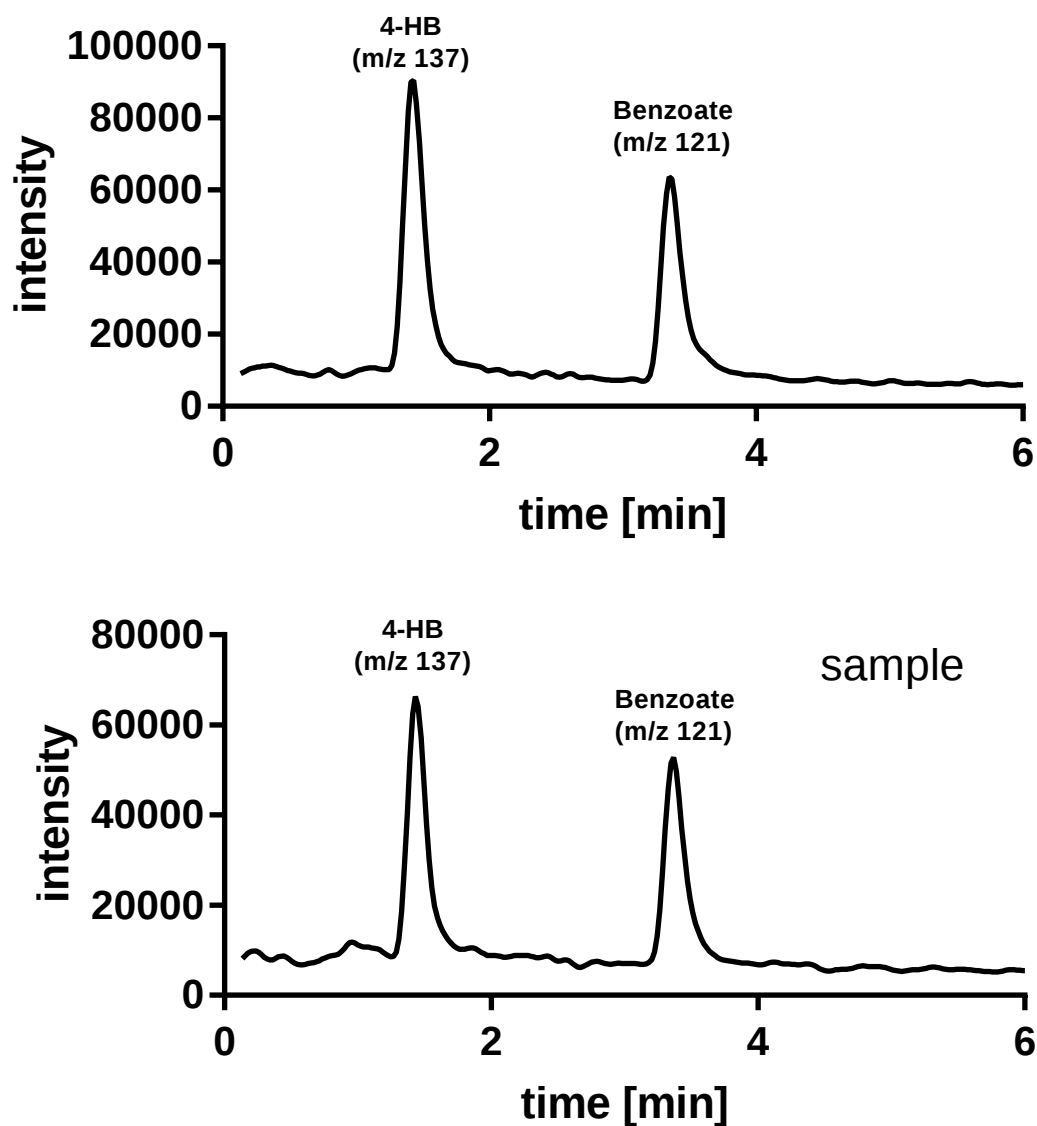
**Additional File 1: Fig. S3. LC-MS analysis of microbially produced PC**

The concentration of PC ( $m/z = 153$ , negative mode) produced by the constructed *C. glutamicum* strains was analyzed by LC-MS analysis in the culture supernatant. The retention times of the identified peaks were identical to the retention times of commercially available standards of the respective compound (upper chromatogram). Benzoate ( $m/z 121$ ) was used as an internal standard.



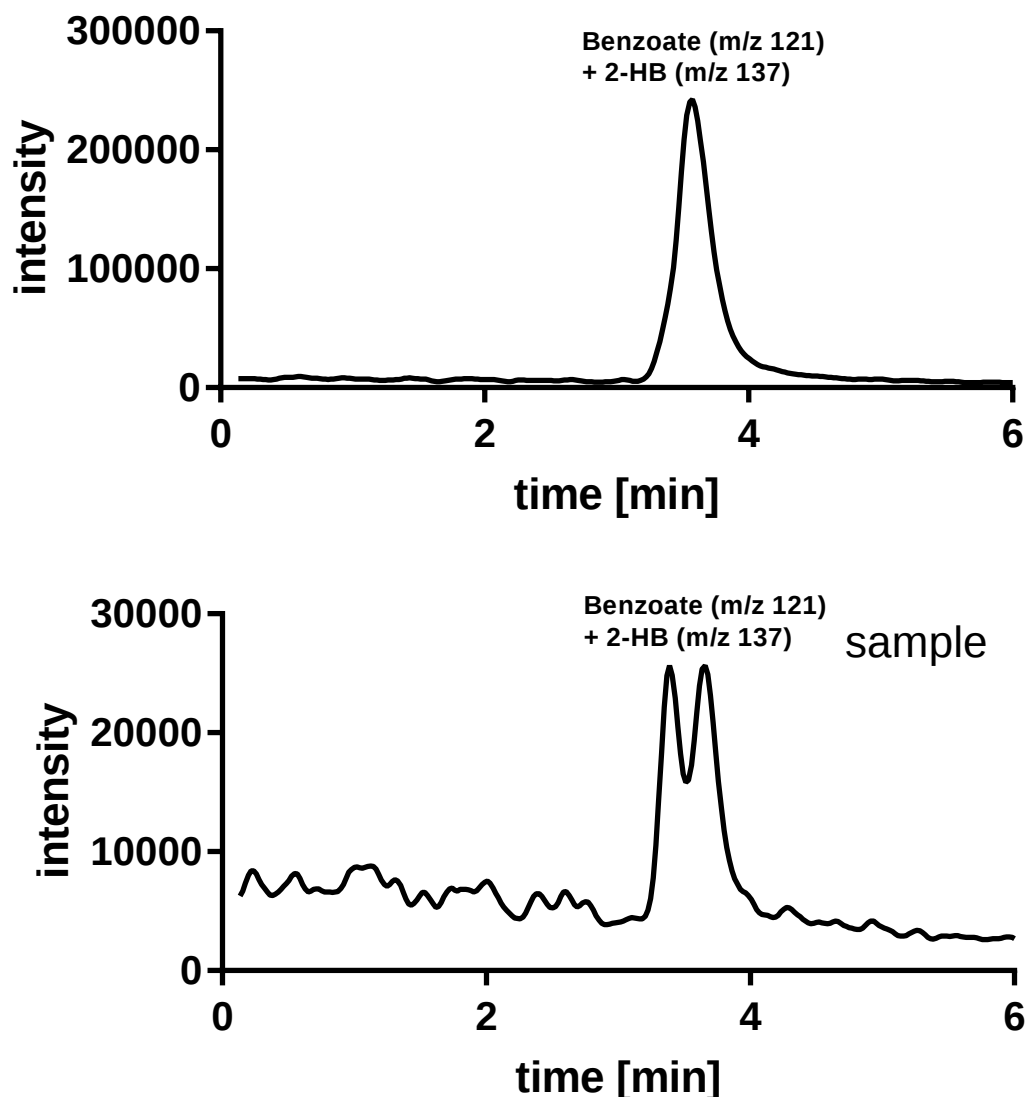
**Additional File 1: Fig. S4. LC-MS analysis of microbially produced 3-HB**

The concentration of 3-HB ( $m/z = 137$ , negative mode) produced by the constructed *C. glutamicum* strains was analyzed by LC-MS analysis in the culture supernatant. The retention times of the identified peaks were identical to the retention times of commercially available standards of the respective compound (upper chromatogram). Benzoate ( $m/z 121$ ) was used as an internal standard.



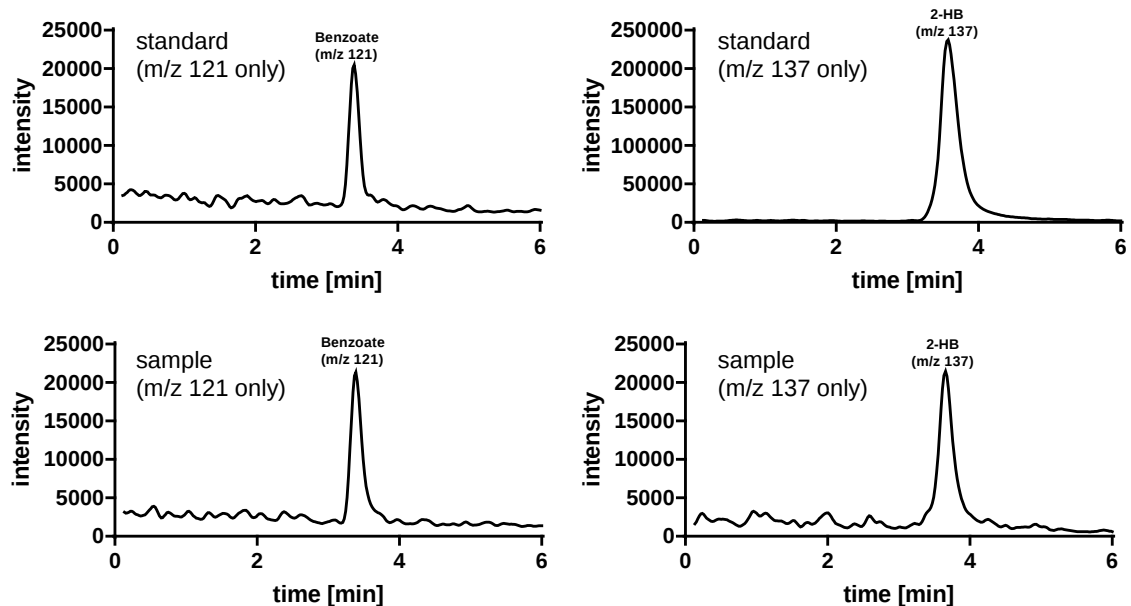
**Additional File 1: Fig. S5. LC-MS analysis of microbially produced 4-HB**

The concentration of 4-HB ( $m/z = 137$ , negative mode) produced by the constructed *C. glutamicum* strains was analyzed by LC-MS analysis in the culture supernatant. The retention times of the identified peaks were identical to the retention times of commercially available standards of the respective compound (upper chromatogram). Benzoate ( $m/z 121$ ) was used as an internal standard.



**Additional File 1: Fig. S6. LC-MS analysis of microbially produced 2-HB**

The concentration of 2-HB ( $m/z = 137$ , negative mode) produced by the constructed *C. glutamicum* strains was analyzed by LC-MS analysis in the culture supernatant. The retention times of the identified peaks were identical to the retention times of commercially available standards of the respective compound (upper chromatogram). Benzoate ( $m/z 121$ ) was used as an internal standard. The retention times of benzoate and 2-HB were very similar so that only one peak was observed during analysis of the metabolite standard. For an unambiguous identification of the respective peaks and accurate calculation of the peak areas extracted chromatograms for  $m/z$  of 2-HB and of benzoate are shown for standard and sample in Fig. S7.



**Additional File 1: Fig. S7. Extracted chromatograms for m/z of benzoate and of 2-HB**

The similar retention times of 2-HB (3.65 min) and of the internal standard benzoate (3.38 min) did not allow an accurate calculation of the peak area. Therefore, the accurate peak areas for these two compounds were obtained from extracted chromatograms for m/z 121 (benzoate) and m/z 137 (2-HB). The merged chromatograms of m/z 121 and 137 yield the obtained chromatogram shown in Fig. S6.