

## Analysis of plant metabolites – the baseline-threshold principle

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## Project objectives

Risk assessment of genetically modified plants (GMP) is investigated based on the background of many years of safe use of traditionally cultivated crops for consumers and environment. Thus every variation within the GMP in contrast to their non-GMP counterpart can refer to a risk and therefore has to be analysed in detail (1).

We establish a GC-MS method for the analysis of approximately 60 compounds from various substance classes (e.g. sugars, sugar alcohols, amino acids, organic acids, fatty acids & sterols) within one extraction step related to the method of Roessner *et al.* in 2000 (2). According to the EFSA guidelines we study the chemical composition of six potato cultivars in comparison to the GMP (3). Thus we will retrieve data from different non-GMPs as a baseline. The results for metabolite analysis of a GMP have then to be compared to this baseline. As a result we can identify uncommon variations in the substance composition of the GMP. The biological relevance has to be proved further.

## From GC-MS data to statistical interpretation of the results

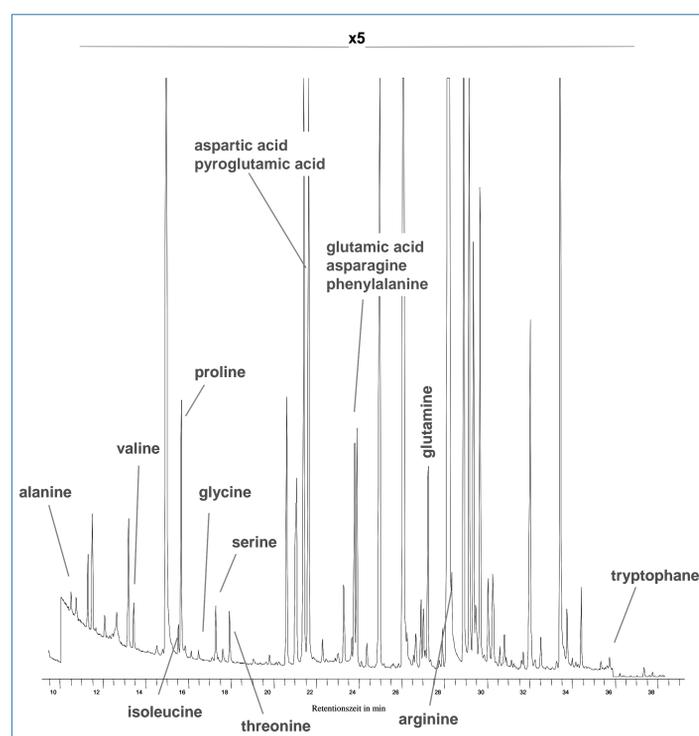


Fig. 1: GC-MS chromatogram with exemplary illustrated compounds

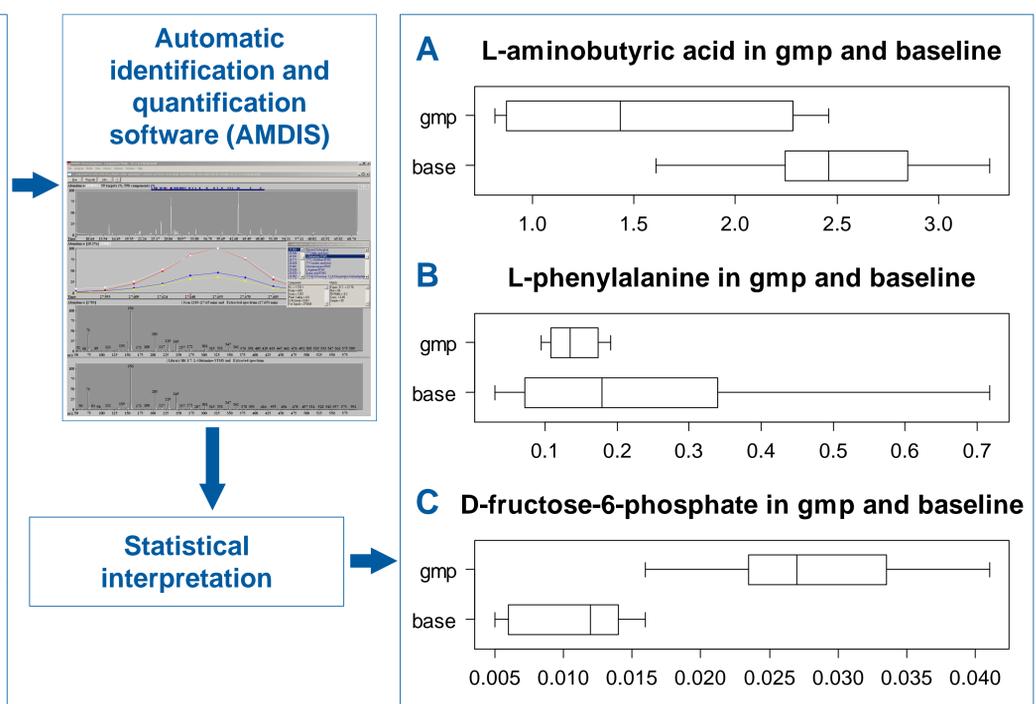


Fig. 2: Comparison of metabolite analysis of a GMP and referring baseline data from non-GMPs (GMP = Albatros PsbY-cphA<sub>6</sub> clon 12; concentration of substances in µmol/mg freeze-dried potato sample)

## Conclusion

For the calculation of metabolite concentrations we use the deconvolution software AMDIS (Automated Mass Spectral Deconvolution and Identification System). With this we can semi-quantify the results of our GC-MS analysis (see **figure 1**). In a second step we combine all the results from isogenic potato lines to build up a so-called baseline which represents the normal variability of single metabolites in potato tubers across different potato cultivars (Albatros, Désirée etc.). Subsequently we are able to compare the metabolite analysis of a GMP with its baseline to confirm substantial equivalence.

**Figure 2** illustrates the results from statistical interpretation of our GC-MS data. Various outcomes for the comparison of GMPs with non-GMPs are possible. In case of L-aminobutyric acid Box plot **A** illustrates the outcome that a substance in a GMP is less than in the base (non-GMPs). Hence no such problems will normally occur for customers. For L-phenylalanine (box plot **B**) the natural variation between different traditional cultivars is much bigger although the concentration for the amino acid is in a normal range. On the other side box plot **C** demonstrates in case of D-fructose-6-phosphate the possibility that the concentration in a GMP is outside the normal variation of the baseline. For detailed risk assessment further analyses like toxicological tests have to be done.

## Literature:

(1) *Guidance document of the Scientific Panel on Genetically Modified Organisms for the risk assessment of genetically modified plants and derived food and feed*, the EFSA Journal (2006) 99: 1-100. (2) Roessner, U., Wagner, C., Kopka, J., Trethewey, R. N., and Willmitzer, L. *Simultaneous analysis of metabolites in potato tuber by gas chromatography-mass spectrometry*. The Plant Journal (2000) 23: 131–142. (3) *Scientific opinion on statistical considerations for the safety evaluation of GMOs*, the EFSA Journal (2010) 8(1): 1250-1309.