Optimization of the "on column" desulfation and gas chromatography of glucosinolates

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Gas chromatography of glucosinolates after their enzymatic desulfation on ion exchange columns is the base of approved methods which are now used in Canada and Germany. Since its first description (THIES 1979 and 1980) obviously no amendments of this technique have been published. In the following we present results of some trials which might be an aid for further improvements of the accuracy and precision of this method.

New three-piece analytical ion exchange column

Until now one-way pipet tips or shortened pasteur pipets have been used as exchanger columns. The drawbacks of these auxiliary devices are well known: Loss of exchanger material due to unproper prepared glas wool plugs, adverse dislocations of the exchanger beds following unskilful pipettations and laborious filling of the columns with a fixed amount of exchanger material. With the column shown in figure 1 these disadvantages can be avoided. The device has been developed and tested in our laboratory and used for all experiments described below.

It consists of a special shaped column with a cone for the uptake of a hypodermic needle (the latter provides a faster flow through of the applied solvents: 170 µl water/min instead of 60 µl water/min without needle) and a funnel with a volume of 1 ml. Spunbonded filters (Paratex I/30) can be mounted on the bottom and the top of the exchanger bed. If DEAE-Sephadex is used only the lower filter can be used ("open bed") because of the shrinking and swelling of this material depending on the ionic strength of the applied solutions.

DEAE-Sepharose Cl-6B as ion exchanger

DEAE-Sepharose has not the before-mentioned disadvantage and can therefore be used in a "closed bed" i.e. with a second filter on the

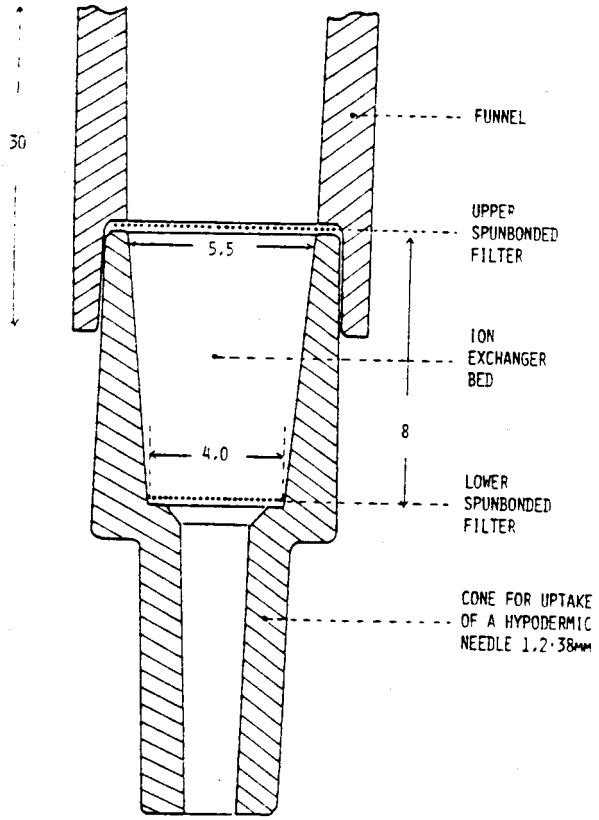


Fig. 1: Design of a three-piece analytical ion exchange column. Supplier: Erich Pollähne, Am Weingarten 14, D-3015 Wennigsen, W.-Germany.

top of the exchanger. This exchange material has a lower but still sufficient capacity for glucosinolates compared with the capacity of DEAE-Sephadex (fig. 2).

A clear advantage of Sepharose is the possibility to use organic solvents as eluents. Primarily experiments have shown that e.g. a solution of 20% n-propanol in water leads to improved elution profiles (fig. 3). Obviously the relatively low tendency of the agarose matrix to nonspecific adsorptions is further reduced by propanol.
On Sepharose the sulfatase has an optimum activity if 100 ul of a 1:20 with water diluted stem solution is used (compare THIES 1980).

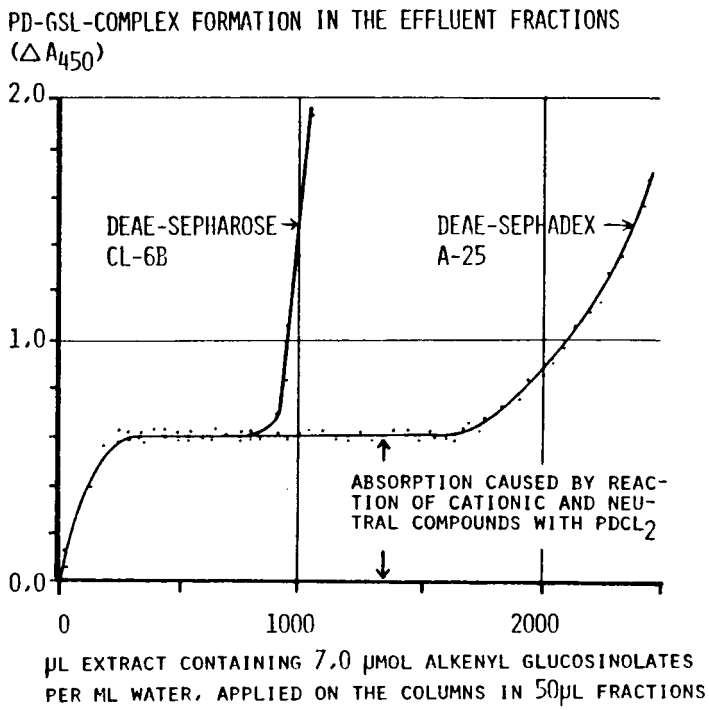


Fig. 2: Break through capacities of DEAE-Sepharose Cl-6B and DEAE-Sephadex A-25 (0.143 ml each) towards rapeseed glucosinolates

Stability of the glucosinolate sulfatase

A long-term experiment has shown that the sulfatase in aqueous solution and stored at -40°C has an extremely good stability (fig. 4). But even as dry powder and stored at +4°C the halflife of the enzyme amounts to 2 years. This means that there are no principal objections towards a central production and distribution of a standardized purified sulfatase which undoubtedly would be a step foreward to the direction of standardization efforts.

Quality control of glucosinolate analyses

Control of the method: Figure 5 illustrates how we tested the performances of columns with different shapes - filled with Sephadex or Sepharose resp. - during our efforts to develop the new three-piece exchanger column: From analyses of stepwise overloaded columns

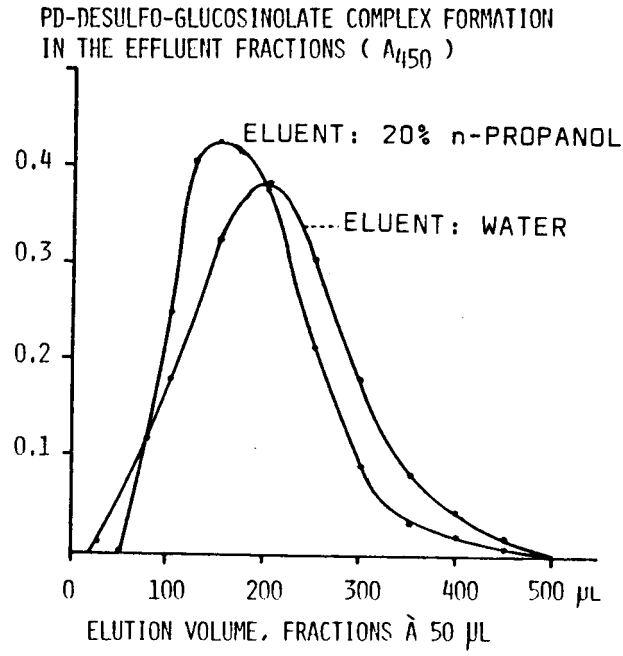


Fig. 3: Elution of desulfo-glucosinolates from a DEAE-Sepharose Cl-6B column: Effect of n-propanol as eluent on the elution profiles.

(normal loading: ca. 0.7 µmol alkenylglucosinolates per 0.143 ml ion exchanger) we calculated values for 4 parameters explained in the text to figure 5. The latter may also be used within serial analyses as quality control parameters, provided a standard seed meal is analysed after e.g. 100 analyses of samples from time to time.

The parameters EL3 and HY1, originally introduced as potential markers of individual rapeseed genotypes (GLAND et al. 1981) have here a second meaning. Constant values for HY1 (standard seed meal) indicate that the two homologues A and B are desulfated, silylated and stable during GLC-separation to the same extent as the homologues C and D.

The slope of the EL3-line is a measure of the quality or the age resp. of the GLC-column. Columns with low separation efficiencies are characterized by low EL3-values. EL3-values calculated from standard seed meal analyses may therefore be used in computer programs. If e.g. an actual EL3-value falls short of a predetermined

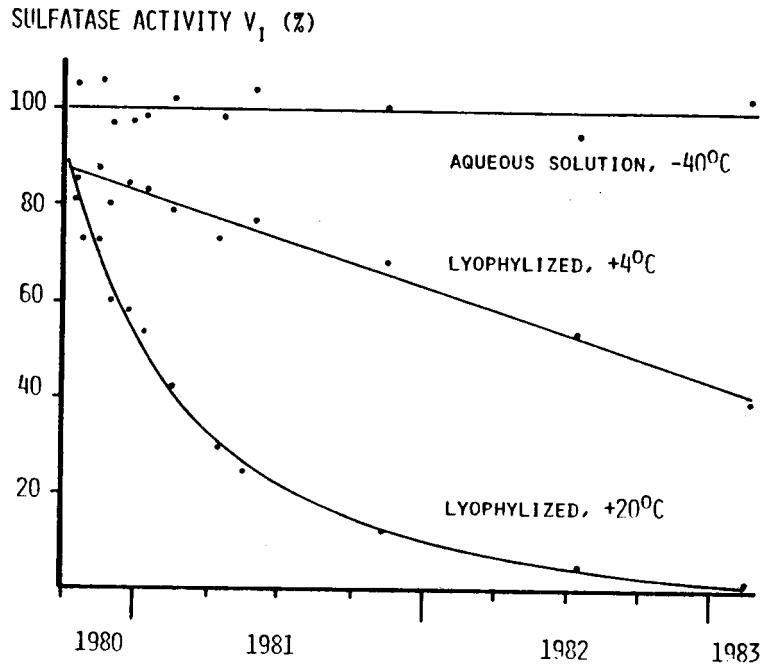


Fig. 4: Storage stability of the glucosinolate sulfatase from Helix pomatia prepared according to THIES (1980). Stored as dry powders at +4°C and +20°C the half-lifes of the enzyme were 2 years and 4 month resp.

border value, a signal may request the technician to replace the column by a new one. The EL3-parameter may also be used as criterion for the selection of the best integration mode: Inject double the amount of TMS-desulfo-glucosinolates which is normally used into the gas chromatograph, calculate the results with a given integration mode and repeat the analysis but using another mode (three are normally offered by the hard-wired GLC computer programs). The integration mode which leads to the highest EL3-value is the best one.

Control of individual analyses: The way we are controlling the precision of the results of individual analyses is explained in figure 6. At present the 10%-tolerance border is valid. For the characterization of the present quality status of the laboratory in Göttingen the following datas may be of interest: 24% of the results from recently performed double estimations for the German Bundessortenamt had absolute ranges which are above the 10%-border.

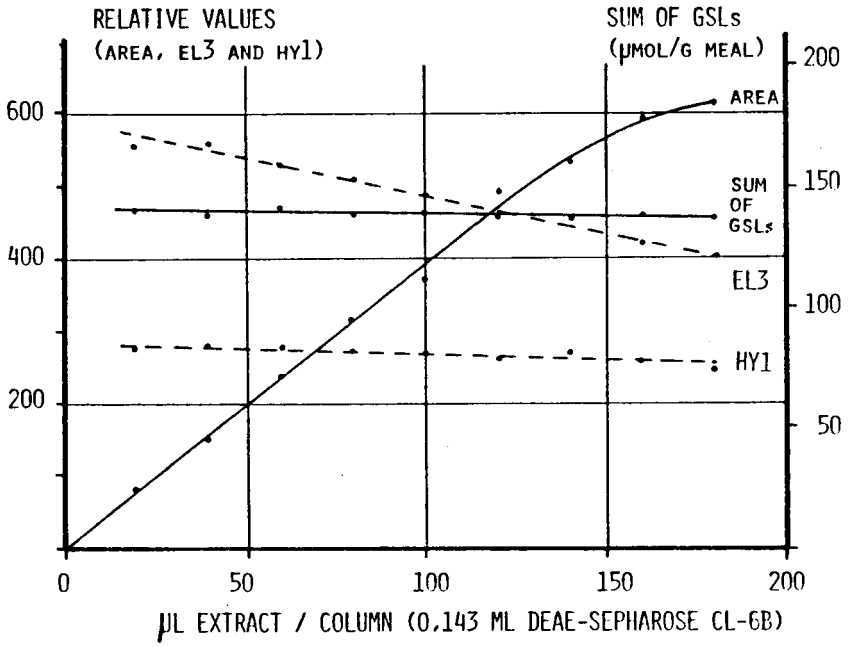
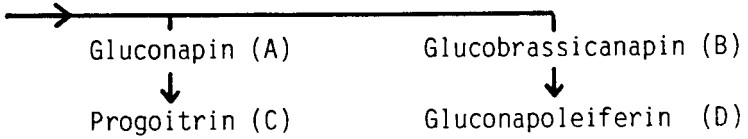


Fig. 5: Quality control of glucosinolate (GSL) analyses. dependence of quality control parameters on the amount of GSLs (0-200 µl extract representing 0-1.4 µmol GSL) which were applied to the ion exchange columns and subsequently analysed by GLC.

Area: Sum of all peak area values ("yield" at the outlet of the GLC columnI, EL3 and HY1: Dimensionless quotients "Products / Substrates": EL3=B+D/A+C, HY1=C+D/A+B.



35% were between the 5%- and 10%-border and 40% of the ranges were under the 5%-border.

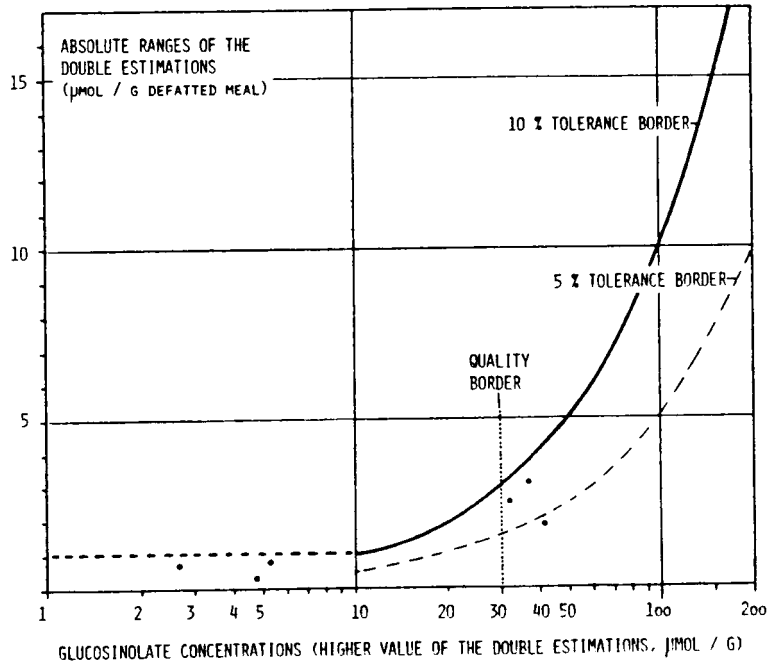


Fig. 6: Control of the precision of glucosinolate analyses in the laboratory in Göttingen. If ranges of double estimations exceed the 10%-border the analyses are repeated.

The inserted points have been taken from the results of 6 analyses which have been performed within a collaborative study on the occasion of this congress.

Literature:

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