Emulation and backtracking of HPLC chromatographic profiles for glucosinolate valuation from total sulphur concentrations in oilseed rape seeds*

Ewald Schnug, Silvia Haneklaus

Abstract

The relationship between the total glucosinolate (GSL) concentration calculated from the total sulfur concentration which had been measured by means of X-ray fluorescence spectroscopy and the content of glucobrassicanapine, glucobrassicine, gluconapine, napoleiferine, progoitrine and 4-hydroxiglucobrassicin in the seeds which were measured by chromatographic methods was determined in the line of quality assessment studies of oilseed rape standard reference materials. The constant ratio between individual aliphatic GSLs which is independent of the total GSL content allows to emulating the concentration of individual GSLs from the total GSL content on basis of the total S content. As indol GSLs represent a constant background value of the total GSL content their estimated concentration is added to the calculated sum of aliphatic GSLs in order to obtain an emulated total GSL content. In a simple program written in BASIC the typical background variability of individual GSLs can be randomly added to the results which yields different chromatograms that are statistically not different from true HPLC chromatograms. This may assist in distinguishing true experimental effects in studies targeting effects on individual GSLs from those of background analytical error variability. The program may also be used for an independent verification of HPLC chromatograms of GSLs in oilseed rape as it allows backtracking of a given total GSL content to its expected individual GSL concentrations in chromatographic analysis.

Key words: Glucobrassicanapine, glucobrassicine, gluconapine, glucosinolates, HPLC, napoleiferine, oilseed rape, progoitrine, sulfur, 4-hydroxiglucobrassicin, X-ray fluorescence, X-RF method

Zusammenfassung

Aus Daten zur Zertifizierung der Gehalte an Schwefel, Gesamt- und Einzelglucosinolaten dreier EU Standardreferenzmaterialien aus Rapssaat und Literaturdaten wurden die Beziehungen der Gehalte zueinander und deren methodisch bedingte Variabilität bestimmt. Kernergebnis ist, dass die Gehalte einzelner Glucosinolate (GSL) eine Funktion des Gesamt-GSL-Gehaltes sind. Dabei ist die

*In memoriam Prof. Dr. Richard Marquardt, Giessen (19.05.1938 – 16.12.2010)
Variability of the single GSLs is higher than that of the total content of GSLs in the sum of all measured values. However, the variability of individual GSLs is significantly higher than the variability of the total GSL content. In this contribution, we present a BASIC program that calculates the variability of individual GSLs. As an extra option, the program permits to add typical variability inherent to chromatographic methods to the calculated variability. This may help to distinguish true experimental effects from background analytical variability.

**Material and Methods**

In general, the relationship between the total sulfur content of rapeseed oilseeds and the total GSL content has been checked excessively during the time the EU was seeking for a proper, fast, and accurate method to distinguish between rapeseed batches of different GSL content for granting subsidies (Schnug, 1988). The breakthrough in terms of accuracy, repeatability, and speed was finally the so-called X-RF (X-ray fluorescence) method. The X-RF method determines the total sulfur concentration in rapeseeds by means of wavelength dispersive X-ray fluorescence analysis in a simple three-step procedure (Schnug and Haneklaus, 1986, 1987a, b). The calculation formulas for computing the total sulfur content from the total GSL content by Schnug and Haneklaus (1988) have been verified in a large number of inter-laboratory comparisons (Schnug and Kallweit, 1987) and these formulas were finally adopted by the EU in combination with wavelength dispersive X-RF as compulsory standard method. The concentration range for total sulfur in rapeseeds is divided into two ranges: above 11 mg/g S the original calibration function of the X-RF method, which has been also verified by stoichiometric assessments (Schnug et al., 1992b, Zhao et al., 1992) is applied (Annex: line 600), but below 11 mg/g S a calibration function considering the non-linear relationship between total sulfur and total GSL content is used (Schnug and Haneklaus, 1990; annex: line 800). This non-linear function compensates slight changes of the total protein concentration in seeds with low GSL concentration due to environmental factors like for instance sulfur deficiency in the growth medium.

In line 920 (annex) a correction factor for systematic error deviations of the HPLC method can be brought into consideration if required. In the recent program description, the adjustment is made to the latest results of EU standardisation.

The analytical data for establishing the regression equations between total GSL content analyzed by X-RF and individual GSL concentration in rapeseed oilseeds were collected from a number of method inter-comparisons conducted by the Bureau of Standards (BCR) of the European Commission (EU) performed on oilseed rape standard reference materials (Schnug et al., 1992; Wathelet et al., 1988, 1991, 1992). The standardized regression equations for calculating individual GSLs from the total GSL content can be found in the program script (see annex) in lines 1000–1600.

During the exhaustive evaluation procedure of the EU standard methods for GSL determination in rapeseeds the urgent need for standard reference materials (SRM)
became an obvious task given to the then operating Community Bureau of Standards (BCR) (WAUSTAFFE et al., 1992; WATTHELET et al., 1988). As a result three SRMs (BCR SRM 190, BCR SRM 366, BCR SRM 367) with certified total contents for GSLs and S were released. Any efforts to certify the concentration of individual GSLs failed utterly. The remarkable phenomenon was that although the sum of 6 individual GSLs analyzed by means of the EU protocol for HPLC was successful, the certification of the individual concentration proved to be not feasible (WATTHELET et al., 1987, 1989). The reason is most likely some methodically inherent incapability of the HPLC to differentiate selectively and sharp enough between individual GSLs.

In the mathematical procedure the variability of the individual GSLs observed in HPLC analysis (WATTHELET et al., 1987) was standardized and randomized (see annex lines 2000–4700), and then added to the individual GSL concentrations calculated from the total S content (see annex lines 5300–5800). In addition to the previously described features the program allows also backtracking of a given total GSL content to its corresponding total S content and based on this data to calculate an expected concentration of individual GSLs (annex line 30000–38735). This procedure is flawed slightly in the lower ranges of GSL concentrations, because the re-calculation is based on an inverted linear calibration function for calculating the total S concentration from GSLs (annex line 34500). This procedure was necessary as the resolution of the cubic function used in line 800 of the annex provided non-conclusive results. This error is, however, well beyond the background error of any HPLC analysis.

**Results and Discussion**

The program “EMU” (see annex), which performs the above described tasks has been written in BASIC 3.11 in an ancient MSDOS 3.2 environment. However, x86 DOS emulators available from the internet still allow to run this kind of program in recent operation systems (e.g. WINDOWS 10). Installation instructions are provided in the annex.

Table 1 displays in 9 steps example runs with “EMU”. The first decision to make is whether a HPLC chromatogram of GSLs in a rapeseed sample shall be emulated from its total S concentration, or if an existing total GSL content in rapeseeds shall be broken down into estimates for individual GSL concentrations (Table 1, step 3). If the first option (HPLC emulation) is chosen another decision has to be made if the results shall be static or with a random variability conform to the common range of HPLC determination (Table 1, step 4). After entering the total S concentration in mg/g S the results are processed either without or with variability (Table 1, steps 6 and 7). It should be mentioned that emulating GSL concentrations from total S analysis requires S determination which is highly accurate and repeatable, features which are only fulfilled by wavelength dispersive X-RF analysis (WAUSTAFFE et al., 1992). Energy dispersive X-RF, combustion methods, spectroscopic analyses and gravimetry following wet digestion of the sample do not comply with the quality standards of wavelength dispersive X-RF (HANNAKELS et al., 1994), hence the quality of the emulated GSL content in terms of accuracy and repeatability will be significantly diminished when S concentrations obtained by these methods are fed into EMU.

In the “without variability” mode the program will provide for a defined S concentration consistently the same GSL concentration. In case the option “with variability” is chosen (Table 1, step 4) then an emulated amount of variability is added to this concentration, which meets the variability for the analysis of individual GSLs assessed during the fruitless certification attempt by BCR (WATTHELET et al., 1987).

Repeated entry of the same S concentration in this mode will generate GSL patterns according to step 6 (Table 1), but with a random amount of variability. For instance: the input of 5 mg/g S at step 5 (Table 1) provides a result of 29.2 μmol/g total GSL when the mode “without variability” had been chosen in step 4 (Table 1); the 10 times repeated input of 5 mg/g S at step 5 gives a series of 31.7, 32.6, 27.0, 26.7, 28.5, 31.7, 28.0, 26.3 and 34.2 μmol/g total GSL. In step 7 the difference between results with and without variability for the particular input at step 4 is shown. With each repetition of the same input of total S the average of the collected results will approximate the value achieved in the mode “without variability”, says for an infinite number of repetitions of the same input for mg/g S the deviation of the averaged emulated results from X-RF in the “with variability” mode (Table 1, step 7) approximates zero. One practical application is to check and verify effects on individual GSLs claimed in variety or growth experiments where no sufficient statistics is provided. Many of such effects reported in the literature (e.g. MARIQUARDT and SCHLESINGER, 1987) fall into the range of uncertainty of the analytical method and thus may become doubtful, at least from a statistical point of view.

Yet another feature of the program is that it permits to backtrack a given total GSL content to its corresponding total S content and to calculate from this data an expected concentration for individual GSLs (Table 1, step 3). An example for the output of the program is given in Table 2. This procedure is flawed a little in the lower ranges of GSL concentration, because the re-calculation is done by employing the inverted linear calibration function for calculating the total S concentration from GSLs (annex line 34500) and as a matter of fact resolving the cubic function used in line 800 of the annex gives non-conclusive results. But this error shall be well beyond the background error of any HPLC analysis.

The “HPLC-check” modus of “EMU” may be used for an independent verification of HPLC chromatograms of oilseed rape GSLs as it allows sourcing a given total GSL content to its expected individual GSL concentrations in a chromatographic analysis.
Table 1. Operational steps and output of the program EMU for the emulation and backtracking of glucosinolates in oilseed rape

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The program is written in BASIC and to run it on any WINDOWS computer first load the emulator software DOSBOX and the BASIC interpreter into the same directory where the script is stored as “EMU.bas”. After starting DOSBOX first key in: keyb gr &lt; return &gt; to activate the German keyboard layout and MOUNT the directory where “EMU.bas” is stored to “C”. The program starts automatically after the command &gt; basic emu &lt; return &gt; is keyed in. Input is case sensitive and accepts only uppercases for alphanumeric inputs.</td>
</tr>
</tbody>
</table>

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To adjust the emulated CPU speed, use ctrl-F11 and ctrl-F12. To activate the keymapper ctrl-F1.

For more information read the README file in the DOSBox directory.

Have fun!

The DOSBox Team http://www.dosbox.com

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Z:\>SET BLASTER = A220 I7 D1 H5 T6
Z:\>keyb gr
Keyboard layout gr loaded for codepage 437
Z:\>mount c f:\pippa\xrf
Drive C is mounted as local directory f:\pippa\xrf\

Z:\>C:.
Directory of C:
EMU BAT 16 08–01–2016 12:36
1 File(s) 16 Bytes.
0 Dir(s) 262,111,744 Bytes free.

C:\>_
Table 1. Fortsetzung

<table>
<thead>
<tr>
<th>DOSBox 0.74, Cpu speed: 3000 cycles, Framskip 0, Programm BASIC</th>
<th>Step</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emulation without variability</td>
<td>6:</td>
</tr>
<tr>
<td>progoitrin: 17.10 umol/g</td>
<td>The program calculates the total GSL content from the total</td>
</tr>
<tr>
<td>napoleiferin: 0.59 umol/g</td>
<td>S content according to the calibration formulas prescribed for</td>
</tr>
<tr>
<td>gluconapin: 7.11 umol/g</td>
<td>employing the X-RF method according to the EU standard</td>
</tr>
<tr>
<td>glucobrassicanapin: 1.27 umol/g</td>
<td>method and the individual GSLs as described in the text.</td>
</tr>
<tr>
<td>4-OH glucobrassicin: 3.04 umol/g</td>
<td></td>
</tr>
<tr>
<td>glucobrassicin: 0.13 umol/g</td>
<td></td>
</tr>
<tr>
<td>total GSL from total S (without var-modus) =: 29.24 umol/g</td>
<td></td>
</tr>
<tr>
<td>Next S input &lt; RETURN &gt; or back to menu (M) or back to system (S)</td>
<td></td>
</tr>
<tr>
<td>Emulation without variability</td>
<td>7:</td>
</tr>
<tr>
<td>progoitrin: 16.57 umol/g</td>
<td>When “2” for results with variability was chosen in the pre-</td>
</tr>
<tr>
<td>napoleiferin: 0.65 umol/g</td>
<td>vious menu, the program adds a random variability to the re-</td>
</tr>
<tr>
<td>gluconapin: 6.89 umol/g</td>
<td>sults which reflects the one observed during ring tests for</td>
</tr>
<tr>
<td>glucobrassicanapin: 1.70 umol/g</td>
<td>individual GSLs with HPLC.</td>
</tr>
<tr>
<td>4-OH glucobrassicin: 3.06 umol/g</td>
<td></td>
</tr>
<tr>
<td>glucobrassicin: 0.39 umol/g</td>
<td></td>
</tr>
<tr>
<td>total GSL from total S (without var-modus) =: 29.25 umol/g</td>
<td></td>
</tr>
<tr>
<td>total GSL from total S (without war-modus) =: 29.24 umol/g</td>
<td></td>
</tr>
<tr>
<td>deviation from X-RF : -0.61 umol/g</td>
<td></td>
</tr>
<tr>
<td>Next S input &lt; RETURN &gt; or back to menu (M) or back to system (S)</td>
<td></td>
</tr>
<tr>
<td>modus: HPLC-create (1) HPLC-check (2)</td>
<td>8:</td>
</tr>
<tr>
<td>****************************************************************</td>
<td>Choosing “2” generates a set of individual GSL concentra-</td>
</tr>
<tr>
<td>total GLS content in seeds (umol/g)</td>
<td>tions expected at the given input of GSL in μmol/g dry (8%</td>
</tr>
<tr>
<td>according to EU HPLC reference method</td>
<td>H₂O) seeds with 42% fat.</td>
</tr>
<tr>
<td>****************************************************************</td>
<td></td>
</tr>
<tr>
<td>Check individual GSL</td>
<td>9:</td>
</tr>
<tr>
<td>progoitrine : 19.09 umol/g</td>
<td>The result is the expected profile of individual GSLs and the</td>
</tr>
<tr>
<td>napoleiferine : 0.66 umol/g</td>
<td>total S content of the seeds.</td>
</tr>
<tr>
<td>gluconapin : 7.94 umol/g</td>
<td></td>
</tr>
<tr>
<td>glucobrassicanapin : 1.42 umol/g</td>
<td></td>
</tr>
<tr>
<td>4-OH glucobrassicin : 3.06 umol/g</td>
<td></td>
</tr>
<tr>
<td>glucobrassicin : 0.13 umol/g</td>
<td></td>
</tr>
<tr>
<td>Checksum : 32.30 umol/g</td>
<td></td>
</tr>
<tr>
<td>Input EU GSL : 33.00 umol/g</td>
<td></td>
</tr>
<tr>
<td>Total S calculated (linear calibration approach)</td>
<td></td>
</tr>
<tr>
<td>(linear calibration approach) : 5.13 mg/g</td>
<td></td>
</tr>
<tr>
<td>Next GSL input &lt;RETURN&gt; or back to menu (M) or back to system (S)</td>
<td></td>
</tr>
</tbody>
</table>
Annex

"EMU" program script for BASIC Interpreters (GW-BASIC 3.11, or newer)
The following program emulates HPLC analyses according to the EU standard method from total sulfur analyses in rapeseeds. The program is written in BASIC and to run it on any WINDOWS computer first the emulating software DOSBOX (DOSBOX, 2016) and a BASIC (GW-BASIC, 2016) interpreter have to be loaded in the same subdirectory, where the script is stored as “EMU.bas”. The script below must be copied from line 10–40000 and saved in plain ASCII as emu.bas in the same directory as the basic interpreter and a batch file “emu.bat” which contains one line with the command “basica emu” in. After starting DOSBOX first key in: keyb gr < return > to activate the German keyboard layout and then use the BASIC command “MOUNT” to access the directory where your program files are stored.

The program itself starts after the command > basic emu < return > is keyed in. The input is case sensitive and accepts only upper cases for alphanumeric inputs.

If you are unable to retrieve the script in ACII from this file you can download it from this source (DOI: 10.13140/RG.2.1.1128.8089): https://www.researchgate.net/publication/293146193_Emulation_and_backtracking_of_HPLC_chromatographic_profiles_for_glucosinolate_valuation_from_total_S_concentrations_in_oilseed_rape_Executable_BASIC_program_in_ASCII

The program supports no printer, if hardcopies are required simply SNIPE the result tables and print directly from the SNIPE program provided with the operating system.

Table 2. Backtracking individual GSLs in oilseed rape seeds from the total GSL content and comparison of backtracked GSLs with individual GSLs emulated from the total S content by means of the program “EMU”

<table>
<thead>
<tr>
<th>Glucosinolate</th>
<th>Backtracking* from 33 μmol/g total GSL (μmol/g)</th>
<th>Emulation** from 5 mg/g total S (μmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progoitrine</td>
<td>19.1</td>
<td>18.1</td>
</tr>
<tr>
<td>Napoleiferine</td>
<td>0.66</td>
<td>0.63</td>
</tr>
<tr>
<td>Gluconapine</td>
<td>7.94</td>
<td>7.6</td>
</tr>
<tr>
<td>Glucobrassicanapine</td>
<td>1.42</td>
<td>1.4</td>
</tr>
<tr>
<td>4-OH-glucobrassinic</td>
<td>3.06</td>
<td>3.1</td>
</tr>
<tr>
<td>Glucobrassicin</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>Sum</td>
<td>32.3</td>
<td>30.1</td>
</tr>
</tbody>
</table>

* Program “EMU” Modus “HPLC-check”
** Program “EMU” Modus “HPLC-create, without variability”
96 INPUT" ******************************************************>>: ", MODUS
97 IF MODUS = 2 GOTO 10000
98 IF MODUS = 1 GOTO 190
190 CLS
194 PRINT: Print: PRINT
195 PRINT" with variability no (1) or yes (2) "
196 INPUT" ******************************************************>>: ",VAR
210 CLS
214 PRINT: PRINT: PRINT
240 PRINT" total sulphur content in seeds (mg/g) "
250 INPUT" ******************************************************>>: ",S
260 CLS
400 REM GSL calculation according to NCS- or linear system
500 IF S < 10.9999 GOTO 800 ELSE 600
600 RFA = 14.99* S-43.87
700 GOTO 900
800 RFA = –5.6* S + 2.8*(S* S)-.12*(S* S* S)+3.5
900 REM basic calculations for single glucosinolates
910 REM correction to BCR level status may 1990
920 RFA = RFA*.974+.15
1000 REM Routines derived from program > singel.sps < updated for BCR results
1100 PRO=((.71492* RFA)-4.4234)*.886 + 2.11
1200 GNL=((.0073* RFA)+.46634)* 2.838–1.351300 GNA=((.23856* RFA)-.74791)* 1.111+.023
1400 GBN=((.04147* RFA)+.08421)* 1.18-.29
1500 OH4=((.00069* RFA)+4.2658)* 8–31.25
1600 GBC=((-.00293* RFA)+.35497)*-.179+.177
1700 GSLTOT = (PRO + GNL + GNA + GBN + OH4 + GBC)
1800 REM
2000 REM random functions: time factor bevore rnd = (2* standard deviation)
2100 REM of deviation predicted from measured values)* 10; minus sd* 10 (mean = 0)
3000 REM variability for progoitrine
3100 RANDOMIZE((2211/1000)*VAL(MID$(TIME$,4,2))* VAL(RIGHT$(TIME$,2))
3200 VARPRO=((INT(72.72* RND(1)+1))-36.36)/10
3300 REM variability for napoleiferine
3400 RANDOMIZE((1710/1000)*VAL(MID$(TIME$,4,2))* VAL(RIGHT$(TIME$,2))
3500 VARGNL=((INT(18.92* RND(1)+1))-9.46)/10
3600 REM variability for gluconapine
3700 RANDOMIZE((79/10)*VAL(MID$(TIME$,4,2))* VAL(RIGHT$(TIME$,2))
3800 VARGNA=((INT(18.26* RND(1)+1))-9.16)/10
3900 REM variability for glucobrassicanapine
4000 RANDOMIZE 19834100 VARGBN=((INT(17.48* RND(1)+1))-8.74)/10
4200 REM variability for 4-hydroxy glucobrassicin
4300 RANDOMIZE 1959
4400 VAR4OH=((INT(29.49* RND(1)+1))-14.86)/10
4500 REM variability for glucobrassicine
4600 RANDOMIZE((1954/1000)*VAL(MID$(TIME$,4,2))* VAL(RIGHT$(TIME$,2))
4700 VARGBC=((INT(6.76* RND(1)+1))-3.38)/10
4800 REM selection create or check modus
4900 IF MODUS = 1 GOTO 5000
4950 IF MODUS = 2 GOTO 31000
5000 REM selection with or without variability from line 196
5100 IF VAR = 1 GOTO 8191
5200 IF VAR = 2 GOTO 5300
5300 PROV = PRO + VARPRO
5400 GNLV = GNL + VARGNL
5500 GNAV = GNA + VARGNA
5600 GBNV = GBN + VARGBN
5700 OH4V = OH4 + VAR4OH
5800 GBCV = GBC + VARGBC
5910 IF PROV < 0 THEN PROV = .01
5920 IF GNLV < 0 THEN GNLV = .01
5930 IF GNAV < 0 THEN GNAV = .01
5940 IF GBNV < 0 THEN GBNV = .01
5950 IF OH4V < 0 THEN OH4V = .01
5960 IF GBCV < 0 THEN GBCV = .01
6000 REM output with variability
6100 PRINT "Emulation with variability"
6197 PRINT
6200 PRINT "progoitrin: "; PRINT USING "###.##"; PROV; PRINT " umol/g"
6300 PRINT "napoleiferin: "; PRINT USING "###.##"; GNLV; PRINT " umol/g"
6400 PRINT "gluconapin: "; PRINT USING "###.##"; GNAV; PRINT " umol/g"
6500 PRINT "glucobrassicanapin: "; PRINT USING "###.##"; GBNV; PRINT " umol/g"
6600 PRINT "4-OH glucobrassicin: "; PRINT USING "###.##"; OH4V; PRINT " umol/g"
6700 PRINT "glucobrassicin: "; PRINT USING "###.##"; GBCV; PRINT " umol/g"
6800 GSLV = PROV + GNLV + GNAV + GBNV + OH4V + GBCV
6900 PRINT
6950 PRINT "total GSL from total S (with var-modus) =: "; PRINT USING "###.##"; GSLV; PRINT " umol/g"
6970 PRINT "total GSL from total S (without var-modus) =: "; PRINT USING "###.##"; GSLTOT; PRINT " umol/g"
7000 DEVV = GSLV - RFA
7100 PRINT "deviation from X-RF: "; PRINT USING "###.##"; DEVV; PRINT " umol/g"
7200 PRINT
7300 PRINT
7400 INPUT "next S input < RETURN > or back to menu (M) or back to system (S) ", X$ 7500 PRINT
7600 IF X$ = "M" THEN 90
7700 IF X$ = "S" THEN 40000
7800 GOTO 210
7900 CLS
8000 REM output without variability
8010 IF PRO < 0 THEN PRO = .01
8020 IF GNL < 0 THEN GNL = .01
8030 IF GNA < 0 THEN GNA = .01
8040 IF GBN < 0 THEN GBN = .01
8050 IF OH4 < 0 THEN OH4 = .01
8060 IF GBC < 0 THEN GBC = .01
8100 CLS
8191 PRINT "Emulation without variability"
8195 PRINT
8200 PRINT "progoitrin: "; PRINT USING "###.##"; PRO; PRINT " umol/g"
8300 PRINT "napoleiferin: "; PRINT USING "###.##"; GNL; PRINT " umol/g"
8400 PRINT "gluconapin: "; PRINT USING "###.##"; GNA; PRINT " umol/g"
8500 PRINT "glucobrassicanapin: "; PRINT USING "###.##"; GBN; PRINT " umol/g"
8600 PRINT "4-OH glucobrassicin: "; PRINT USING "###.##"; OH4; PRINT " umol/g"
8700 PRINT "glucobrassicin: "; PRINT USING "###.##"; GBC; PRINT " umol/g"
8750 PRINT
8800 PRINT "total GSL from total S (without var-modus) =: "; PRINT USING "###.##"; GSLTOT; PRINT " umol/g"
8900 PRINT
9000 INPUT "next S input < RETURN > or back to menu (M) or back to system (S) ", X$ 9612 PRINT
9700 IF X$ = "M" THEN 90
9800 IF X$ = "S" THEN 40000
References

DOSBOX, 2016: DOSBox, an x86 emulator with DOS. http://www.dosbox.com/.


MARIQUARDT, R., V. SCHLESINGER, 1985: Methodische Untersuchungen zur Glucosinolatbestimmung bei Raps. Fette Seifen Anstrich-
Ewald Schnug and Silvia Haneklaus, Emulation and backtracking of HPLC chromatographic profiles for glucosinolate ...