METHODICAL INVESTIGATIONS ON GLUCOSINOLATE DETERMINATION OF OILSEED RAPE

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INTRODUCTION

After rapeseed production in Germany and the EC was restricted to 00-quality different methods for glucosinolate determination were employed by the different registration authorities.
In particular, semiquantitative tests for preliminary analysis, three different national laboratory methods and the European arbitral method[1)] were in utilization.
In this study the results of the different methods are compared and critically evaluated.

MATERIAL and METHODS

Ten rapeseed strains with glucosinolate (GSL) contents between 14 µmol and 75 µmol GSL/g airdried seed were selected from breeding material of the institute by X-ray fluorescence spectroscopy. This material was analyzed by the following methods:

00-Dip-Test: according to Thies et al. (1987) the glucose, liberated by autolysis of glucosinolates was determined by teststrips (Clinistix, Ames Miles GmbH, No. 2844). Classification of the samples was done after assessment of colouring by several persons.

Palladium-test (Pd-test) with intact glucosinolates: the procedure described by Thies (1982) was slightly modified. The extinction of the Pd/GSL-complex was measured at 450 nm with a Titertek Miniskan 330.

Palladium-test with desulfo-glucosinolates: the GSLs were desulfatated on column (DEAE Sephadex A-25) with 1% H1-sulfatase (Sigma S-9626) and the effluent was measured at 425 nm after colouring by $PdCl_2$-solution.

Estimation of the Pd-tests was done by using calibration curves.

Glucose-method (GLUCO-M): the measurement of the enzymatically liberated glucose was carried out according to Fiebig (1988) with the hexokinase-D-glucose-UV-test (Boehringer, No. 716251) (Fiebig, 1988) at 340 nm against a blank which takes into consideration the different concentrations of free glucose in rapeseed.

X-ray fluorescence spectroscopy (XRF): the procedure described by Schnug and Haneklaus (1987) was used with an Oxford OX 1017 X-ray-spectrometer equipped with Rh-excitation and argon/methane detector-flushing.

Isothermal gaschromatography (GLC-iso): the analysis was carried out on defatted samples by the procedure described by Thies (1978):
-desulfatation on column (DEAE Sephadex A-25) with H1-sulfatase (Sigma S-9626)

1) till 1990 temperature-programmed GLC

-GLC-parameters: Fractovap, type 4300 (Carlo Erba Strumentazione) with FID; 2 m glas column packed with 2% OV 7 on Chromosorb W AW, 80-100 mesh;
injector and detector 300°C, oven 220°C;
carrier gas 30 ml N_2/min.
The alkenyle-GSLs gluconapin, glucobrassicanapin, progoitrin and gluconapoleiferin were determined by this method. The results were converted into µmol GSL/g airdried seed.
Temperature-programmed gaschromatography (GLC-tp): GLC-tp was carried out by LUFA[2] Kiel following the European arbitral procedure of that time[1] (Amtsblatt der EG, No. L 256/43, Anhang VIII). The indole-GSl glucobrassicin and 4-hydroxy-glucobrassicin are recorded in addition to the alkenyle-GSLs. As for GLC-iso the results were converted into µmol GSL/g airdried seed.

HPLC-method (HPLC)[3]: sample preparation and analyses were performed according to the national prescription (Amtsblatt der EG, No. L 256/43, Anhang VIII) with a Philips PYE UNICAM-system on a reversed phase column ET 200/8/4 Nucleosil 5 C_{18}.

Statistical evaluation was carried out with the statistical package STATGRAPHICS (STSC Inc.).

The 95% confidence intervals and coefficients of variation were calculated for all methods. In addition, the correlation coefficients and the relative deviations from the mean values were calculated between each two methods, respectively.
The Pd-tests were compared with the quantitative methods. Correlation coefficients and standard deviation for accuracy related to compared method were determined for proportion of conformity.
The relationship between GLC-tp results of the samples and their classification through 00-Dip-test by different persons was described with Pearson's coefficient of contingency (Köhler et al., 1984).

RESULTS

Means of glucosinolate content through quantitative measurement are listed in table 1.

Table 1. Glucosinolate contents (µmol/g airdried seed)

sample	GLC-tp	GLC-iso	HPLC	XRF	GLUCO-M
1	9.4	4.5	14.2	15.2	9.0
2	12.7	9.7	17.0	14.6	6.7
3	12.8	9.8	22.1	21.0	18.7
4	18.9	14.4	19.4	18.5	17.8
5	20.2	18.6	21.0	24.3	19.6
6	20.9	17.4	28.9	26.0	14.2
7	22.2	19.1	28.7	30.4	15.5
8	24.3	22.9	28.7	27.3	23.5
9	95.4	96.7	77.6	72.5	64.8
10	96.8	97.3	76.5	72.3	80.6

2) Landwirtschaftliche Untersuchungs- und Forschungsanstalt
3) since 1990 European arbitral procedure

Table 2 shows the statistical parameters for the different methods.

Table 2. Statistical parameters of quantitative methods

	V (µmol/g)	C 95% (µmol/g)	cv %
GLC-tp	1.34	17.27	4.01
GLC-iso	2.70	5.98	5.91
HPLC	2.76	4.15	4.65
XRF	2.56	5.95	5.46
GLUCO-M	4.35	6.26	12.95

V = range, C 95% = 95% confidence interval, cv% = coefficient of variation

The best reproducibility in this investigation is achieved by HPLC. In 95% of all cases a difference of 4.15 µmol GSL/g airdried seed is not exceeded between measurements of single samples. Similar results were obtained by Buchner (1987) and in an investigation carried out by the AFNOR[4] in 1989. The reproducibilities of GLC-iso (5.98 µmol/g), XRF (5.95 µmol/g) and GLUCO-M (6.26 µmol/g) are slightly better and remarkably superior for GLC-tp (17.27 µmol/g).
The lowest coefficient of variation is found for GLC-tp (4.01%) followed by HPLC with 4.65%. The coefficients for GLC-iso (5.91%) and XRF (5.46%) are in similar order. Whereas the GLUCO-M shows a high relative deviation for measurements of a single sample with a coefficient of variation of 12.95%. In spite of the comparatively low reproducibility, this is caused by the relatively low GSL-contents which were obtained by this method.

The analysis of variance indicates that all methods produce significantly (α = 0.05) different results. The estimated correlation coefficients and the relative deviation of means are listed in table 3.

Table 3. Comparison of quantitative methods

	GLC-iso		**HPLC**		**XRF**		**GLUCO-M**	
	r	$\%M_d$	r	$\%M_d$	r	$\%M_d$	r	$\%M_d$
GLC-tp	0.9996	21.8	0.9922	24.8	0.9908	25.0	0.9804	24.4
GLUCO-M	0.9804	29.2	0.9694	33.8	0.9716	31.8		
XRF	0.9916	43.3	0.9964	7.9				
HPLC	0.9925	43.5						

r = correlation coefficient
$\%M_d$ = relative deviation of means

4) Association Française de Normalisation, ISO/TC 34/SC 2 N 380E

Although clear differences are conspicuous between individual values (Table 1) high coefficients of correlation are obtained. Differences between the methods are described better by the relative deviation of means.
The best concordance exists between HPLC and XRF (r = 0.9964, $\%M_d$ = 7.9%). Therewith the results of Schnug and Kallweit (1987) are confirmed.
Evident are the considerable differences between GLC-iso and other methods. That may be explained largely by indole-GSL-content not detectable with this method (Landerouin et al., 1987); this fraction may amount to 40% of total GSL-content (Marquard and Schlesinger, 1985).
Concordance between GLC-tp and HPLC is also insufficient with regard to deviation of means (24.8%). Results of GLC-tp in our investigation tend to yield lower values as compared to the results described by Schnug (1988). This effect is explained in the literature by insufficient detection of indole-GSLs (Buchner, 1987; Slominski and Campbell, 1987). In addition, the defatting step before gaschromatographic analysis may be the cause for losses of glucosinolates (Wathelet, 1987). Hence, we assume in agreement with Buchner (1987) and Spinks et al. (1984) that results of HPLC are more reliable than results of GLC-tp.
Accordance of the `glucose-method' as compared others is poor in our investigation. The largest differences are found between `glucose-method' and HPLC ($\%M_d$ = 33.8%) in spite of the high correlation (r = 0.9694). The values measured by the `glucose-method' are clearly lower than that by HPLC or XRF as described by Schnug and Haneklaus (1988) too. Comparatively good results were obtained with the palladium-test in parts of the investigation. Respective statistical parameters are listed in table 4.

Table 4. Statistical parameters of Pd-tests

	Palladium-test					
compared method	intact glucosinolates			desulfoglucosinolates		
	C 95%	sd_a	r	C 95%	sd_a	r
GLC-tp	9.86	9.39	0.9853	9.17	6.64	0.9957
GLC-iso	10.22	9.78	0.9866	5.77	11.40	0.9972
HPLC	7.27	6.31	0.9744	7.02	3.91	0.9948
XRF	6.84	6.09	0.9758	6.51	3.54	0.9957
GLUCO-M	8.22	9.16	0.9652	7.88	7.64	0.9739

C 95% = 95% confidence interval (µmol/g), sd_a = standard deviation for accuracy (µmol/g), r = correlation coefficient

The reproducibility of Pd-test with desulfo-GSL was better than that of Pd-test with intact GSL, independent of calibration procedure.
The correlations to the quantitative reference methods are very high between r = 0.9739 and r = 0.9957 and are in accordance with results of Schnug and Haneklaus (1986) and

Thies (1982). However standard deviation for accuracy is sufficient only in some cases.
Large differences of more than 9 µmol GSL/g airdried seed were found between Pd-test of intact glucosinolates and GLC-procedures as well as the 'glucose-method'. Better concordance exist of Pd-test of intact GSLs to HPLC and to XRF. The discrepancy of about 6 µmol/g is acceptable for a rapid method. Consequently calibration of the Pd-test by HPLC leads to more reliable quantitative results than calibration by GLC-iso (Marquard and Schlesinger, 1985).
Conformity of Pd-test with the quantitative reference method is improved by desulfatation before measurement. Bjerg and Sörensen (1987) obtained better results by Pd-test with desulfo-GSL, too. This improvement is mainly explained by purification by ion exchange during sample preparation.

Calculation of results obtained by 00-Dip-test revealed that classification (table 5) depends upon the real GSL-content by only about 75%. Consequently 25% of classification result from subjective differences in personal judgement.

Table 5. Classification of samples by 00-Dip-test

sample	absolute frequency			**GLC-tp**
	<25 µmol	≈30 µmol	>40 µmol	µmol/g
1	5			9.4
2	4	1		12.7
3	3	2		12.8
4		3	2	18.9
5		5		20.2
6		5		20.9
7	1	4		22.2
8		2	3	24.3
9			5	95.4
10			5	96.8

Only 23 single judgements of a total of 50 were in accordance with real proportion classes obtained by GLC-tp. 27 single judgements resulted in the upper class. The 00-Dip-test was too insensitive, especially within the range 10 - 30 µmol GSL/g airdried seed. Samples were classified 19 times into the class near 30 µmol/g and 5 times in the class of more than 40 µmol GSL/g airdried seed although they included less than 25 µmol/g as measured by GLC-tp. Classification of samples corresponding to their GSL-content measured by GLC-tp was done correctly only in 13 cases within this range into the class lower than 25 µmol/g.

CONCLUSIONS

Comparison between HPLC, XRF and the GLUCO-M as well as GLC-iso and GLC-tp revealed that all methods result in significantly different results.
Introduction of HPLC as a European arbitral procedure is supported by the results of this investigation. The best reproducibility and the assumed correctness of measured values favours HPLC for official use. Moreover, HPLC is more

informative than all other methods because of detection of all main individual glucosinolates in rapeseed and separate representation of their proportions.
XRF is proposed for practical reasons because of security and rapidity of measurement. The possibility to calibrate XRF by HPLC and the resulting conformity of measured values as well as the comparatively simple preparation of samples and the rapid measurement favoures XRF for practical purposes.
For a pre-assortment of rapeseed charges the 00-Dip-test seems to be of limited use. Numerous incorrect judgements have to be presumed because of a lot of possible sources of error and the eminent influence of the results by subjective personal judgement. Further, the 00-Dip-test proofed to be rather insensitive within the critical range of 10 - 30 µmol GSL/g airdried seed.
Better results are obtainable by the Pd-tests, where calibration by HPLC is recommended. Desulfatation as a step of clean-up before measurement improves conformity between Pd-test and quantitative methods as well as reproducibility of measured values.
The results of this investigation show that it should be feasible to establish a quantitative photometric method for determination of glucosinolates in rapeseed based on the Pd-test with desulfo-glucosinolates.

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