# SIMULTANEOUSLY DETERMINATION OF GLUCOSINOLATES AND THEIR BREAKDOWN PRODUCTS BY MEANS OF HPLC

B. RUDOLPH

Agricultural Institution of Thuringia, Laboratory Department, Naumburger Str. 98, D-07743 Jena, Germany

## ABSTRACT

Common HPLC-methods for determination of glucosinolates exclud degradation products in the step of sample preparation. This may be dangerous in animal nutrition, because of potential health riscs of these substances in several rapeseed containing concentrates. An ion-pair reversed phase HPLC method with simple sample preparation procedure was tested for investigation of several rapeseed materials with different treatment.

## INTRODUCTION

The determination of glucosinolates (GSL) in rapeseed has been well established for many years. HPLC of Desulfo-GSL (Anonym 1990) is the preferred method for identification of individual glucosinolates. Otherwise total GSL content can be determined by means of sulfur analysis coupled with a suitable model including a regression function (Schnug et al. 1992).
But there are no valid methods for the determination of GSL or their degradation compounds in rapeseed products like ground seed, defatted seed, rapeseed meal or compound feed.
Starting from two rapeseed qualities (0 and 00) we prepared several products with different degrees of GSL degradation under defined conditions. This samples were characterized by determination of total sulphur content, of glucose release after ion exchange (Lein and Schön 1969) and HPLC of Desulfo-GSL (Anonym 1990). The aim of this paper was the comparision of these results with a new developed method for the simultaneous determination of GSL and degradation products.

## EXPERIMENTAL

### 1. Sample treatment

Two different rapeseed qualities with low (00) and high (0) GSL contents were treated as described in Table 1, in order to simulate several possible ways of GSL degradation. Defined amounts of water were added to create sufficient conditions for reaction of myrosinase.

### 2. Extraction procedure

Amounts of approximately 0.2 g were extracted stepwise with 10 ml boiling methanol/water (70:30) to inactivate myrosinase. A suitable alternative is the extraction with hot methanol/water in an ultrasonic bath.
These extracts were directly injected into the HPLC system after centrifugation at 5000 rpm for 10 minutes.

TABLE 1. Treatment and declaration of rapeseed samples

| Treatment | 00-quality | 0-quality |
|---|---|---|
| without | A 1 | B 1 |
| grinding | A 2 | B 2 |
| 1:1 water, freeze drying | A 3 | B 3 |
| 1:1 water, 60°C drying | A 4 | B 4 |

## 3. Conditions for HPLC-measurements

The usual HPLC procedures separate the glucosinolates from other compounds by anion exchange, i.e. nonionic degradation products will be excluded. One way to analyze both groups simultaneously is the ion-pair reversed phase HPLC, published by Helboe et al. (1981) for determination of individual GSL. We modified the chromatographic conditions for separation of GSL and degradation products in rapeseed and treated samples (Table 1).

Column: NUCLEOSIL 100 $C_{18}$ 5 µm (KNAUER)
250 mm x 4 mm
Eluent: 73 % 50 mM $K_2HPO_4$
+ 1 mM Cetyltrimethylammoniumhydrogensulfate

27 % Acetonitril
(all reagents from MERCK)
Flow: 1 ml/min
Detection: λ=235 nm

## 4. Standard substances and peak identification

Solutions of sinigrin (ROTH) and glucotropaeolin (MERCK) in 70% Methanol were used to optimize the chromatographic conditions. This procedure is time consuming because of the long equilibration times in ion-pair HPLC. The chromatograms of the rape products were compared with results of three reference rapeseed materials (BCR-Nr. 190, 366, 367) for identification of individual GSL. This identification was proofed by recording the UV-spectra of GSL peaks with a photodiode array detector.
Methanolic (70%) solutions of allylisothiocyanate (ROTH), goitrin (HERAEUS) and indol-3-acetonitril (FLUKA) were used to receive some chromatographic information about theoretical degradation products.

## RESULTS

The main glucosinolates (approximately 90 % of total content) progoitrin (PRO), gluconapin (GNA) and 4-hydroxyglucobrassiciin (4OH) are used as "indicator" components for degradation during several treatment procedures. Table 2 shows the con-

tents of these glucosinolates in comparision to their total content determined by means of a modified glucose method (Lein and Schön 1969).
In the chromatograms of the treated samples two unidentified peaks appear, which are probably generated by degradation products. The peak at a retention time of about 4.5 min increases very strongly with decreasing GSL content (peak areas in Table 2). The identification was impossible up to now, but the UV-spectra seems to be very similar to an indolyl compound.

Table 2. GSL content in treated rapeseed in µmol/g
Peak areas of the unknown compound in absolute units

| Sample | PRO | GNA | 4OH | Σ | Glucose | unknown Peak-A. |
|---|---|---|---|---|---|---|
| A 1 | 8.2 | 3.2 | 2.1 | 13.5 | 14.0 | 6 |
| A 2 | 8.0 | 3.4 | 2.0 | 13.4 | 13.8 | 12 |
| A 3 | 4.8 | 2.3 | 0.6 | 7.7 | 13.0 | 19 |
| A 4 | 1.4 | 1.4 | n.d. | 2.8 | 3.8 | 140 |
| B 1 | 43.8 | 19.0 | 2.9 | 65.7 | 82.2 | 5 |
| B 2 | 44.8 | 19.5 | 2.4 | 66.7 | 83.5 | 10 |
| B 3 | 37.0 | 16.5 | 2.1 | 55.6 | 46.5 | 16 |
| B 4 | 1.4 | n.d. | n.d. | 1.4 | 3.2 | 21 |

n.d.: not detectable

ACKNOWLEDGEMENTS

The author thanks Dr. Schöne (Jena) for careful preparation of the different rapeseed samples and Dr. Lange (Potsdam) for his support in comparative GSL determination.

REFERENCES

Anonym (1990). Oilseeds. Determination of glucosinolates by high performance liquid chromatography. *Official Journal of the European Communities*, L 170, 03.07.27-34

Helboe, P., Olsen, O. and Sørensen, H. (1980). Separation of glucosinolates by high performance liquid chromatography. *Journal of Chromatography*, 197, 199-205

Lein, K.A. and Schön,W.J. (1969). Quantitative determination of glucosinolates in brassica halfseeds. *Angewandte Botanik*, 43, 87-91

Schnug, E., Haneklaus, S., Zhao, F. and Evans, E.J. (1992). Relations between total sulphur and glucosinolate content in rapeseed-calibration of the X-RF method. *Fat Science & Technology*, 94, 246-249