SEPARATION OF INTACT GLUCOSINOLATES BY HPLC AND STUDY ON THE CHARACTERISTICS OF GLUCOSINOLATES IN INDIAN MUSTARD

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ABSTRACT

Elimination of nutritionally toxic glucosinolates continues to be of immense concern for breeding of '00' varieties of rapeseed and mustard. Data are lacking on the status of individual glucosinolates for different Indian mustard cultivars. In the present study a HPLC method was developed for the separation and determination of individual glucosinolates. The status of individual glucosinolates in different Indian mustard cultivars was determined using this method.

INTRODUCTION

Glucosinolates being anionic in nature, can be separated by Reverse Phase - High Performance Liquid Chromatography (RP-HPLC) as desulphated compounds or as intact moeity using either ion-pairing reagents (Helboe *et al.*, 1980) or specific salts in aqueous mobile phase (Borje and Hase, 1988). The desulfotechnique is highly specific but some glucosinolates can not be determined and it requires time consuming enzymatic desulphatation (Bjerg and Sorensen, 1987a). Separation of intact glucosinolate by ion pairing reagents requires elevated column temperature and costly ion pairing chemicals (Bjerg and Soresen, 1987 b). The use of specific salts in aqueous mobile phase eliminates all these disadvantages. This paper describes the method developed for the separation of intact glucosinolates in rapeseed-mustard seed extract using specific salt in mobile phase and status of individual glucosinolates in Indian mustard cultivars.

EXPERIMENTAL

Separation of intact glucosinolates by RP-HPLC

Intact glucosinolates were extracted from dried (45°C overnight), crushed seeds (100 mg) by hot water (3 x 1 ml) in a screw capped centrifuge tube placed on a boiling water bath for 15 min. Extract was filtered through 0.22 μm membrane and 10μl sample was injected into HPLC (Waters LC Module I gradient liquid chromatograph equipped with RP-18 column and UV-VIS detector) via the autosampler and peaks were monitored at 229 nm. The peaks were identified on the basis of relative retention time and quantified by caliberations developed for individual glucosinolates.

Figure 1. shows that the separation of all the major glucosinolates is achieved by this simple method within 10 minutes.

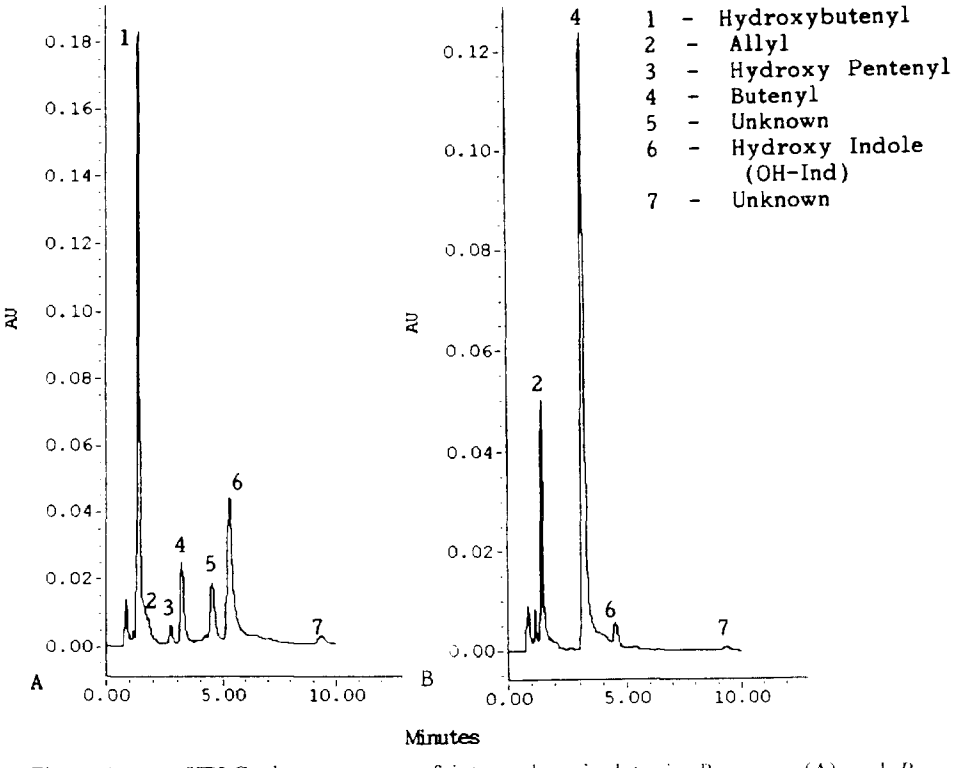

Figure 1. HPLC chromatogram of intact glucosinolate in *B. napus* (A) and *B. juncea* (B)

Status of individual glucosinolates in Indian mustard

Table 1 shows the total glucosinolate content in Indian mustard cultivars. Allyl glucosinolate varied from 6.94 to 32.47 μmole reflecting wide variations among cultivars. Butenyl component is also found to vary widely ranging from 24.74 to 52.55 μmole. Thus Indian cultivars seems to be high butenyl type. The total glucosinolate content ranges from 37.17 to 87.14 μmole. Thereby none of the cultivar was found low in total glucosinolate.

Table 1 Glucosinolate composition of Indian mustard (*B. juncea*) cultivars

Cultivar	Glucosinolate content (μmole/g dried seed)			
	Allyl*	Butenyl*	Oh-Ind*	Total**
Pusa Bold	7.07	35.52	2.72	45.31
Varuna	6.94	29.53	1.79	38.26
Kranti	32.47	52.55	2.12	87.14
Krishna	9.86	24.74	2.57	37.17
RH-819	16.29	31.73	2.12	50.14
RLM-198	19.52	36.49	2.18	58.19
RH-30	8.36	26.82	2.19	37.37
RLM-619	12.01	34.49	2.21	48.71
RLM-100	17.74	26.36	3.38	47.48
Laha T-59	12.88	34.43	2.90	50.21

* Mean of 3 replications

** Total of mean values inclusive of OH-Ind

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