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HPTLC Quantitation of Sinigrin from Seed Powder of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.)

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A simple and sensitive high performance thin layer chromatographic (HPTLC) method has been developed for the quantitative determination of sinigrin from seed powder of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.). The seeds of both the species were extracted using a hydro-alcoholic mixture and the extracts were separated on HPTLC Silica gel 60 F_{254}, using a mobile phase of ethyl acetate:methanol:water (6:2:1). Detection and quantitation were carried out densitometrically at λ = 230 nm. The method was validated in terms of linearity, precision and accuracy. Linear response to sinigrin was obtained over the concentration range of 400-1200 ng/band. The method proposed for quantitative monitoring of sinigrin from seed powder of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) is simple and accurate and can be used for routine quality testing.

Key Words: Sinigrin, *Brassica juncea* (L.) Czern, *Brassica nigra* (L.), High performance thin layer chromatographic, Quantitation.

INTRODUCTION

Brassica juncea (L.) Czern and *Brassica nigra* (L.) belong to family Cruciferae[1]. *Brassica juncea* (L.) Czern commonly called brown mustard or Indian mustard is cultivated chiefly in India in the states of Bihar, Haryana, Madhya Pradesh and West Bengal[1]. *Brassica nigra* (L.) commonly called black mustard is native to the Mediterranean and is grown in India in Punjab, Delhi, Uttar Pradesh, Madhya Pradesh[1]. The seeds of both the species contain glucosinolates, which are rich in sulphur containing glycosides[2] and are characteristic of the plants of genus *Brassica*[3].

Among the glucosinolates, sinigrin(2-propenyl or allylglucosinolate) is predominantly found in the seeds of both the species of *Brassica*[3]. Recent research has focused on the anticarcinogenic activity of glucosinolates[2]. Sinigrin is a precursor of anticancer compound called allyl isothiocyanate[4]. Allyl isothiocyanate can stimulate phase II enzymes such as glutathione-S-transferases in the body to detoxify carcinogens[2]. It is also used as a powerful antifungal compound[5].

These spices are used as food ingredients and hence there is a need to determine the level of sinigrin as a measure of quality in these widely used spices.

A literature survey reveals that a TLC method is reported for identification of sinigrin in seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.)[6]. A HPLC method is reported for the simultaneous analysis of sinigrin and allyl isothiocyanate

in mustard bran and mustard seeds[7]. Sinigrin has been determined in *B. juncea*, *B. nigra*, *B. oleracea*, *B. rapa* and *B. napus* using a simple extraction method combined with ion-pair HPLC analysis[2]. Also, sinigrin has been determined in mustard seed without desulfatation by reverse phase ion-pair liquid chromatography[5]. A near-infrared spectroscopic method for screening individual and total glucosinolate contents in Indian mustard seeds has been reported[8].

However, there is no validated HPTLC method for the quantitative determination of sinigrin in the seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.). The method proposed in the present research work can be used for routine quality control of the seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.).

EXPERIMENTAL

Ethyl acetate and methanol used were of analytical grade and were obtained from E. Merck (Mumbai, India). Distilled water was used for analysis. Sinigrin (a monohydrate potassium salt) was purchased from Sigma-Aldrich Chemie (Steinheim Germany) and had a purity of 99 %. Aluminium backed HPLTC silica gel 60 F_{254} plates of 0.2 mm thickness were purchased from Merck (Mumbai, India).

Authentic seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) were provided by National Bureau of Plant Genetic resources (NBPGR, Pusa, New Delhi) (accession nos. IC 241636 and IC 417804-A, respectively). The seeds of both the species were powdered. Each powder was passed through a BSS No. 85 mesh sieve. Each sample powder was stored in a separate air-tight container at room temperature (28 ± 2 ºC).

Preparation of stock and working standard solutions of sinigrin: A stock solution of sinigrin (1 mg/mL) was prepared by transferring accurately weighed 10 mg of standard sinigrin to 10 mL standard volumetric flask. 1 mL of water was added to it to dissolve the standard and the volume was made upto the mark with methanol. 1 mL of this stock solution was transferred to a separate 10 mL standard volumetric flask. Volume of the flask was made upto the mark with methanol (0.1 mg/mL).

Preparation of sample solutions: Accurately weighed 500 mg of seed powder of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) was transferred to a 10 mL standard volumetric flask. 1 mL of water was added to each flask, followed by 5 mL of methanol. Each flask was sonicated for 0.5 h, followed by intermediate shaking. After cooling to room temperature (28 ± 2 ºC), contents of each flask were made upto the mark with methanol. Each sample solution was then filtered separately through Whatman No. 41 filter paper and the filtrate of each sample solution was collected. For the determination of sinigrin, the sample solutions were diluted 1:5 with methanol.

Chromatographic conditions: Chromatography was performed on 20 cm × 10 cm aluminium backed HPTLC silica gel 60 F_{254} plates (E. Merck). The standard and samples were applied as sharp bands of 8 mm length by means of Camag

(Muttenz, Switzerland) automatic TLC sampler 4(ATS-4), equipped with 25 μL Hamilton syringe. They were applied at a distance of 8 mm from bottom, the distance from the edges was 15 mm. The delivery speed of application was 200 nL/s.

The plates were developed to a distance of 70 mm using a mobile phase of ethyl acetate:methanol:water, 6:2:1 (v/v) using Camag glass twin trough chamber. After development, the plates were dried in a current of air at ambient temperature. The plates were scanned by means of Camag TLC scanner 3, with winCATS software, version 1.4.4., in absorbance-reflectance mode using deuterium lamp at $\lambda = 230$ nm.

Validation of the HPTLC method

Linearity of detector response: For determination of linearity, 4, 6, 8, 10 and 12 μL volumes of sinigrin standard solution (0.1 mg/mL) corresponding to 400-1200 ng/band of sinigrin were applied to 20 cm × 10 cm HPTLC plate. The plates were developed and scanned as described above. The detector response to different concentrations of standard sinigrin was measured. A graph of peak area of sinigrin against corresponding concentrations of sinigrin was plotted. The graph was linear over the concentration range of 400-1200 ng/band of sinigrin and correlation coefficient(r) was 0.998.

Limit of detection (LOD) and limit of quantitation (LOQ): The limit of detection and limit of quantitation were determined at a signal-noise ratio of 3:1 and 10:1, respectively. The LOD and LOQ values for sinigrin were found to be 50 and 170 ng/band, respectively.

Precision: The method was validated for instrumental precision, repeatability and intermediate precision. Instrumental precision was carried out by repeated analysis (n = 10) of standard sinigrin containing 800 ng/band. The results were expressed as per cent RSD of peak area. Repeatability of the method was checked by preparing three different concentrations (250, 750 and 1000 mg/mL) of sample solutions of seed powder of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.). Each sample was prepared as described earlier. Each concentration of both the samples were applied in triplicate, on same day and analyzed by the above procedure. Intermediate precision was also carried out in a similar manner, but on three different days. The values of per cent RSD for each of the above parameters were found to be below 2, indicating the method is precise to carry out the analysis.

Specificity: The specificity of the proposed HPTLC method was ascertained by overlapping UV spectra of standard sinigrin with UV spectra of sinigrin in the two *Brassica* species (Fig. 1). The sinigrin bands in both the samples were compared at three positions, the peak start, peak middle and peak end. There was excellent correlation between all spectra obtained at each of the three position of bands. Thus, the peak of sinigrin was not masked by the peak of any other component in the sample which was indicative of peak purity.

System suitability: The system suitability tests were carried out to confirm that system used is adequate to carry out analysis and gives reproducible results.

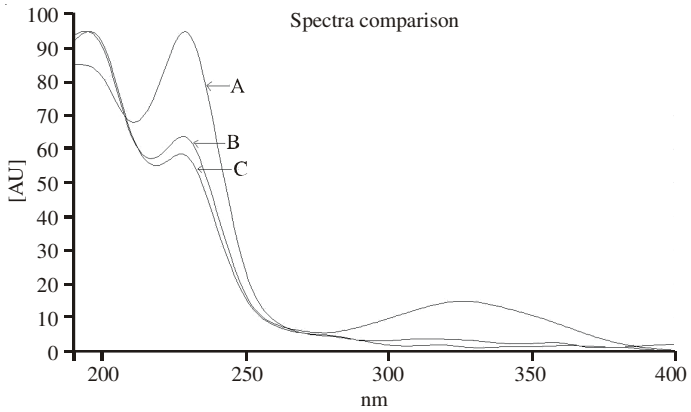

Fig. 1. UV spectra obtained from sinigrin standard (A); sinigrin present in *Brassica nigra* (L.) (B) and *Brassica juncea* (L.) Czern (C)

The parameters that were studied to evaluate the suitability of the system were peak area values and retardation factors (R_f) of sinigrin. The system suitability was carried out by applying standard sinigrin containing 800 ng to the plates six times. The plates were developed under optimized chromatographic conditions. The results were expressed as per cent RSD. The values of per cent RSD were found to be below 2, indicating that the method is reproducible and hence can be used for routine chromatographic analysis.

Accuracy: Accuracy of the method was checked by performing recovery experiment from the seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.), by standard addition method, at three levels. For determination of recovery from *Brassica juncea* (L.) Czern, known amounts of sinigrin (1.07, 1.61 and 2.15 mg) were added to 500 mg of seed powder of *Brassica juncea* (L.) Czern. For determination of recovery from seeds of *Brassica nigra* (L.), known amounts (3.1, 4.65 and 6.62 mg) were added to 500 mg of seed powder of *Brassica nigra* (L.). Each sample was extracted as described earlier and each extract was analyzed seven times by the developed HPTLC method. The amount of sinigrin recovered from both the samples was determined at each level. The per cent recovery was determined. The results of recovery analysis of seed powder of *Brassica juncea* (L.) and *Brassica nigra* (L.) are given in Table-1.

Estimation of sinigrin from *Brassica juncea* (L.) Czern and *Brassica nigra* (L.): Seven replicates of seed powder solution of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) (13 µL for *Brassica juncea* (L.) Czern and 4 µL for *Brassica nigra* (L.) (sample preparation as described before) were analyzed by the developed HPTLC method. Peak area values obtained for sinigrin in the sample solutions of both the species were measured. The amount of sinigrin from each of the two species of *Brassica* were determined from the calibration curve. The results obtained from assay are given in Table-2.

TABLE-1
RESULTS OF RECOVERY ANALYSIS OBTAINED FROM SEED POWDER OF
Brassica juncea (L.) Czern AND *Brasica nigra* (L.)

Plant	Level	Amount of sample weight (mg)	Amount of standard added (mg)	Mean amount found (mg)[a]	Recovery (%)
Brassica juncea (L.) Czern	0	500.3	0.00	2.15 ± 0.051	98.8
	1	500.2	1.07	3.18 ± 0.056	
	2	500.3	1.61	3.70 ± 0.063	
	3	500.7	2.15	4.67 ± 0.069	
Brassica nigra (L.)	0	500.8	0.00	7.75 ± 0.043	98.2
	1	500.4	3.10	10.65 ± 0.052	
	2	500.2	4.65	12.17 ± 0.055	
	3	500.5	6.22	13.75 ± 0.061	

a: Mean ± SD (n = 7).

TABLE-2
RESULTS OBTAINED FROM ASSAY OF
Brassica juncea (L.) Czern AND *Brassica nigra* (L.)

	Brassica juncea (L.)	*Brassica nigra* (L.)
Mean weight of sample (mg)	500.5	500.3
Mean amount of sinigrin found in sample (mg)[a]	2.150 ± 0.045	7.750 ± 0.038
Percentage RSD of peak area of sinigrin	0.65	0.62
Mean amount of sinigrin found (%)	0.43	1.55

a: Mean ± SD (n = 7).

RESULTS AND DISCUSSION

The TLC method reported for identification of sinigrin from seeds of *Brassica juncea* (L.) and *Brassica nigra* (L.) uses a mobile phase of *n*-butanol:*n*-propanol: acetic acid:water (3:1:1:1) (v/v)[6]. The mobile phase reported is time-consuming; as the development time required is 1 h 15 min. The mobile phase used in the present research work comprises of ethyl acetate:methanol:water (6:2:1) (v/v). The development time required is 20 min.

Reversed phase (RP)-HPLC has generally been the method of choice in recent years for quantitation of glucosinolates in mustard samples. The present HPTLC method is simple and rapid and analysis can be made with considerable saving of time and at low cost.

The HPTLC method developed is specific as it is able to resolve sinigrin efficiently from the other components present in extracts of both the samples. The R_F value of sinigrin was found to be 0.48. Typical chromatograms obtained from standard sinigrin and sinigrin present in the two species of *Brassica* are shown in Fig. 2.

The quantitation is carried out at $\lambda = 230$ nm as it is the wavelength of maximum absorption of sinigrin. The values of per cent RSD for instrumental precision, repeatability and intermediate precision were found to be below 2, which indicates that the method is precise to carry out the analysis. The per cent recovery values for

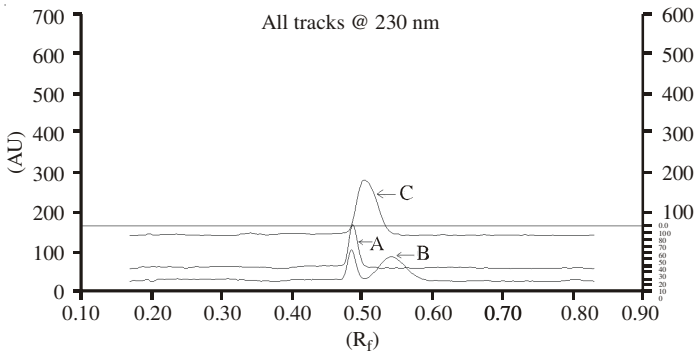

Fig. 2. Overlay of high performance thin layer chromatographic chromatogram of sinigrin standard (A) and sinigrin present in *Brassica juncea* (L.) Czern (B) and *Brassica nigra* (L.) (C)

sinigrin from seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) were found to be 98.8 and 98.2 %, respectively, indicating good accuracy of the method.

Conclusion

The high performance thin layer chromatographic method developed for the quantitative determination of sinigrin from seeds of *Brassica juncea* (L.) Czern and *Brassica nigra* (L.) is simple, rapid and accurate and can be used as routine quality control method.

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