

Application and Evaluation of Ion-Exchange UV Spectrophotometric Method for Determination of Sinapine in *Brassica* Seeds and Meals

Shawn X. Wang,[†] B. Dave Oomah,^{*,†} and D. Ian McGregor[‡]

Pacific Agri-Food Research Centre, Agriculture and Agri-Food Canada, Summerland, British Columbia V0H 1Z0, Canada, and Agriculture and Agri-Food Saskatoon Research Centre Saskatoon, Saskatchewan S7N 0X2, Canada

A cation-exchange column packed with CM-Sephadex C25 matrix was evaluated for its efficiency, capacity, elution volume, and reproducibility in purifying crude extracts of rapeseed or meal for the determination of sinapine content. Combined with a single extraction and ion-exchange column purification procedure, sinapine content in the seed or meal could be quantified with a coefficient of variance of 3.9%. Recovery of sinapine from rapeseed or meal using this method was 95–114% with a lower limit of the sinapine detection of 2.5 µg/mL. The proposed method is not labor-intensive and is applicable to batch analysis of up to 120 samples per day, thereby reducing the cost of analysis.

Keywords: Sinapine determination; *Brassica*; rapeseed meal; cation-exchange column

INTRODUCTION

Sinapine (Figure 1) is one of the major phenolic choline esters in oil-extracted rapeseed meal (Krygier et al., 1982; Clausen et al., 1982; Bouchereau et al., 1991) and is responsible for the bitter taste and poor palatability of rapeseed meal when fed to domestic animals as a protein source (Ismail et al., 1981; Andersen, 1985; Andersen and Sorensen, 1985). A quantitative analytical method for the determination of sinapine is required not only for nutritional analysis but also for plant breeders for selection of low sinapine varieties and for the food/feed industries. Several quantitative methods have been developed for sinapine. These include the Reinecke salt method (Tzagoloff, 1963; Austin and Wolff, 1968), the titanium tetrachloride method (Ismail and Eskin, 1979), and analytical techniques, such as UV spectrophotometry (Legueut et al., 1981; Clausen et al., 1983, 1985), gas chromatography (Krygier et al., 1982) and high-performance liquid chromatography (Strack, 1980; Henning, 1982; Clausen et al., 1983). It has been shown that when CM-Sephadex C25, a cation-exchange resin, is introduced to purify methanol extracts of rapeseed meal for sample preparation prior to analysis, the accuracy of sinapine determination is increased (Clausen et al., 1982; Bjerg et al., 1984). UV spectrophotometric detection combined with an ion-exchange purification procedure (IE-UV method) is a promising quantitative analytical method.

The efficiency of the CM-Sephadex C25 column and conditions for its application have not been reported. The objectives of the present study were (1) to simplify the procedures for sinapine extraction from meal, (2) to determine the capacity, elution conditions, and efficiency of the CM-Sephadex C25 ion-exchange column

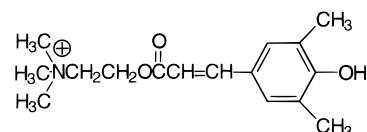


Figure 1. Chemical structure of sinapine.

for extract purification; and (3) to evaluate the accuracy and reproducibility of this analytical method.

MATERIALS AND METHODS

Materials. All chemicals were of reagent grade or better and purchased from the Fisher Scientific Chemical Co. (Nepean, ON, Canada) with the exception of CM-Sephadex C25 cation-exchange resin (40–120 µm), which was obtained from the Aldrich Chemical Co. (Milwaukee, WI). A Poly-Prep column was purchased from Bio-Rad Laboratories (Mississauga, ON, Canada). Sinapine was isolated and purified as sinapine bisulfate from the seeds of *Sinapis alba* L. cv. Ochre, on the basis of the method of Clandinin (1961). Eighty rapeseed meal samples, including 20 strains and cultivars of *Brassica napus* L., *B. rapa* L., *B. juncea* L., *B. nigra* L., *B. carinata* L., and *Sinapis alba*, used for evaluating analytical method, were from the Agriculture and Agri-Food Canada Saskatoon Research Centre and prepared using an oilseed crusher as described by Raney et al. (1987). Four meals from *B. napus* cultivars Midas-1 (certified seed, lot 79-7535407-41), Midas-2 (Saskatoon Research Centre standard 179-52), R-500 (research line, Saskatoon Research Centre seed collection) and Low-Ochre-1 (artificially prepared by ethanol extraction of meal of the *S. alba* cv. Ochre) were used to determine the efficiency and capacity of CM-Sephadex C25 cation-exchange columns.

Preparation of CM-Sephadex C25 Ion-Exchange Column. The pretreatment of CM-Sephadex C25 resin was according to the manufacturer's instruction. Ten grams of resin was added to 200 mL of distilled water; the resin was allowed to swell for 8 h. Swollen resin was transferred to a 25 × 400 mm glass column and washed with 2 L of 1 N HCl, followed by water until the pH of the eluate was neutral. Treated resin (0.6 mL) was packed by gravity into a Bio-Rad Poly-Prep column (8 × 40 mm with a 10-mL reservoir) and settled with water washing to form a gel bed of about 14 mm in height.

* To whom correspondence should be addressed [telephone (250) 494-6399; fax (250) 494-0755; e-mail OomahD@em.agr.ca].

[†] Pacific Agri-Food Research Centre.

[‡] Agriculture and Agri-Food Saskatoon Research Centre.

Preparation of Crude Extract of Rapeseed Meal (Single-Extraction Procedure). Oil-extracted air-dried meal (50 mg) was weighed into a 15-mL screw-top glass test tube, 5 mL of 70% aqueous methanol (v/v) was added, and the tubes were capped and then agitated. The meal was extracted in a water bath at 75 °C for 20 min. After cooling, 5 mL of 70% methanol was added to each tube, and the tubes were centrifuged at 1500*g* for 10 min at room temperature. The supernatant was used for sinapine determination.

Sinapine Assay. Clear supernatant (1 mL) was transferred to the prepared CM-Sephadex C25 cation-exchange column. The column was washed with 10 mL of water and eluted with 10 mL of 2 M acetic acid/methanol (1:1 v/v) by gravity. Absorbance of the eluate containing the extracted sinapine was measured at 330 nm against a 2 M acetic acid/methanol (1:1 v/v) blank.

Sinapine Standard Curve. A series of sinapine bisulfate standard solutions was prepared from an aqueous stock solution of sinapine bisulfate (800 µg/mL). Serial dilutions of this stock gave standard solutions of 25, 50, 100, and 200 µg/mL. One milliliter of each solution was applied to the CM-Sephadex C25 column. The column was washed and eluted as described for the meal extract. A standard curve was obtained by plotting $A_{330\text{nm}}$ versus sinapine concentration.

Calculation of Sinapine Content. Sinapine content in the meal was calculated from the standard curve using the equation

$$\text{sinapine (mg/g)} = (\text{ABS} - A)/B(V_t/V_e)(V_{es}/W/1000)$$

where ABS = absorbance of eluate at 330 nm, A = y -intercept (absorbance) of standard curve, B = slope of standard curve (mL/µg), V_t = total volume of extract (mL), V_e = volume of extract applied to column (mL), V_{es} = elution solvent volume (mL), W = weight of meal extracted (g), and 1000 = weight conversion factor (µg/mg).

Efficiency of Meal Extraction. In the multiple-extraction procedure, 2.5 mL of 70% methanol was added to 50 mg of meal. The tubes were capped and agitated. Meal was extracted in a water bath at 75 °C for 10 min, followed by centrifugation at 1500*g* for 10 min at room temperature. The supernatant was transferred to another test tube, and the residue was extracted repeatedly with 2.5 mL of 70% methanol four additional times. The supernatant from each of the five extractions was sampled, and the rest was pooled. The total volume was measured. The sinapine content in each extract and pooled extract was determined as described above.

The effect of extraction time on the completeness of extraction and on the stability of sinapine during extraction was determined at four extraction times (10, 30, 60, and 90 min) with meals of R-500 and Midas-1 by the single-extraction approach.

Capacity of Ion-Exchange Column. Midas-1 meal extract (1 mL) prepared using the single-extraction procedure was transferred onto the first of two columns to determine the capacity of the CM-Sephadex column. The effluent from the first column was applied to the second column. Both columns were washed twice with 10 mL of water. Sinapine was eluted from the second column and assayed as described above.

A second loading and elution cycle was initiated by transferring another 1 mL of the meal extract to the washed first column. The effluent that passed through the first column was again applied to the second column. Both columns were then washed with water, and the sinapine from the second column was determined as described above. The loading and elution cycle was repeated a total of six times. The experiment was repeated four times.

Elution Volume of Ion-Exchange Column. Midas-1 extract (1 mL) prepared using the single-extraction procedure was applied to the CM-Sephadex column. The column was washed with 10 mL of water and then eluted with 1-mL aliquots of 2 M acetic acid/methanol (1:1 v/v). The sinapine concentration in each of the eluates was determined by measuring the absorbance at 330 nm and comparing it to the

standard curve. This allowed the minimum volume of 2 M acetic acid/methanol required to elute the sinapine from the CM-Sephadex column to be determined. The experiment was repeated three times.

Reproducibility of the Ion-Exchange Column. Midas-1 extract prepared using the single-extraction procedure was applied to eight CM-Sephadex C25 columns to test the reproducibility of the CM-Sephadex C25 column for purification of sinapine. The sinapine concentration of the eluate from each column was determined as described above.

Precision and Recovery of the Ion-Exchange-UV Spectrophotometry (IE-UV) Method. The sinapine content of 80 meal extracts prepared using the single extraction procedure was measured in triplicate as described above. Analysis of variance (ANOVA) and coefficient of variation (CV) were used to determine the precision of the method and the variation among the replicates.

Oil-extracted meals of Midas-1 and R-500 and sinapine bisulfate were used to investigate the recovery of sinapine by the IE-UV method. Meals were extracted using the single-extraction procedure as described above except that the first 5 mL of extraction solution included 1 mL of sinapine bisulfate (80 µg/mL 70% methanol). Recovery was calculated using the equation

$$\text{recovery (\%)} = (A - C)/B \times 100\%$$

where A = sinapine content of meal extract with addition of 80 µg of sinapine (µg), B = sinapine content of meal extract without addition of sinapine (µg), and C = 80 µg of sinapine.

RESULTS AND DISCUSSION

Sinapine Standard Curve. Sinapine displayed an absorption maximum at 330 nm. From the standard curve, an extinction coefficient of $1.36 \times 10^{-2} \text{ L } \mu\text{mol}^{-1} \text{ cm}^{-1}$ (calculated as sinapine, MW 310) was obtained. The linear range of measurement was from 0 to 20 µg/mL, with the equation $Y = -0.0027 + 0.0439X$, where Y is the absorbance of sinapine at 330 nm, X is the concentration of sinapine (µg/mL), and -0.0027 and 0.0439 are the intercept and the slope, respectively. The coefficient of determination (R^2) of this equation was 0.999. The lower limit of sinapine detection using this method was determined by measurement of a series of dilution of standard. A greater variation of assay (CV > 10%) was found when the sinapine concentration was <2.5 µg/mL. The pH of eluate was 3.0 in the method. Sinapine stability at this pH was demonstrated by the lack of degradation during 3 days of storage at 4 °C (data not shown).

Efficiency of Sinapine Extraction. To reduce the time required for extracting sinapine from rapeseed meal, the efficiencies of the single-extraction procedure and the multiple-extraction (five times) procedure were compared. Sinapine concentrations measured by using both methods did not differ significantly ($P = 0.05$) (Table 1). The sinapine contents of the Low-Ochre-1 and Midas-2 meals were 11.1 and 24.9 mg/g, respectively, using the single-extraction procedure and 10.3 and 24.9 mg/g, respectively, using the multiple-extraction procedure. In the single-extraction procedure, the methanol extract was not separated from the meal residue after centrifugation. The calculation of sinapine was based on the sinapine concentration in the extract and the volume of methanol originally added to the sample. In the multiple-extraction procedure, the methanol extract was separated from the residue following centrifugation and pooled with several washes. The subsequent calculation of sinapine content was based on the total volume of the pooled extract. In the

Table 1. Efficiency of Extraction and Time Required for Completion of the Single- versus Multiple-Extraction Methods Using Two Meals, Low-Ochre-1 and Midas-2

no. of extraction	time required ^a (min)	sinapine extracted ^b			
		Low-Ochre-1		Midas-2	
		mg/g	%	mg/g	%
multiple-extraction method					
1	100	10.01 ± 1.08	90.9	20.61 ± 2.30	82.4
2		0.92 ± 0.27	8.2	2.90 ± 1.16	11.6
3		0.10 ± 0.06	0.9	0.90 ± 0.28	3.6
4		0.00 ± 0.00	0.0	0.41 ± 0.10	1.6
5		0.00 ± 0.00	0.0	0.20 ± 0.12	0.8
total		11.03	100	25.02	100
pooled extract		10.34 ± 1.28		24.94 ± 1.89	
single-extraction method					
1	30	11.10 ± 1.07		24.90 ± 0.91	

^a Includes extraction and centrifugation. ^b Mean of five replicates.

Table 2. Effect of Extraction Time on Extraction Efficiency Using Two Brassica Meals, R-500 and Midas-1

extraction time (min)	sinapine extracted ^a (mg/g of oil-free meal)	
	R-500	Midas-1
10	11.62 ± 0.28	21.41 ± 2.04
30	11.43 ± 0.86	22.52 ± 1.06
60	11.71 ± 0.21	21.53 ± 0.86
90	10.88 ± 0.74	21.91 ± 1.01

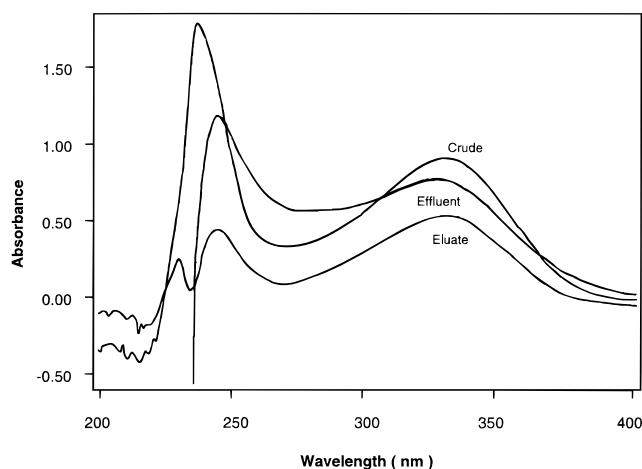
^a Mean of three replicates ± SD.

multiple-extraction procedure, the proportions of sinapine extracted from Midas-2 in each of the five extractions were 82.4, 11.6, 3.6, 1.6, and 0.8%, respectively. For the Low-Ochre-1 meal, the extracted sinapine was 90.9, 8.2, and 0.9% in the first three extractions, respectively, while sinapine was not detected in the fourth and fifth extractions. The two extraction procedures did not give different results, indicating that sinapine has been extracted from the cells in the initial extraction. The sinapine contents in the latter extracts is washed out from the meal residues rather than extracted.

It is desirable to extract sinapine completely from seed or oil-extracted meal in the shortest period and by the simplest procedure to facilitate the analysis of a large numbers of samples. The multiple-extraction procedure has been used to quantitatively extract sinapine from rapeseed meals in the past (Ismail and Eskin, 1979; Clausen et al., 1982; Henning, 1982). Compared to the single-extraction procedure, the multiple-extraction method took >3 times longer to complete. Thus, the single-extraction method was simpler and faster than the multiple-extraction procedure.

Sinapine extraction efficiency did not differ when extraction times ranged from 10 to 90 min (Table 2). These results contradict those of Thies (1991), who suggested that extraction time should not exceed 20 min to avoid sinapine degradation. However, degradation of sinapine was not observed in this experiment when the extraction time for two meal samples, R-500 and Midas-1, was as long as 90 min at 75 °C.

Efficiency of CM-Sephadex C25 Column. A crude methanol extract of Midas-1 meal (5 mg of meal/mL), diluted 10 times with 2 M acetic acid/methanol, had a spectrum with prominent peaks at 238 and 330 nm (Figure 2). When 1 mL of this extract was subsequently passed through a 0.6-mL CM-Sephadex C25 column, the sinapine in the extract was eluted with 10 mL of 2 M acetic acid/methanol. The spectrum of the eluate showed major peaks at 230, 245, and 330 nm, which matched the spectrum of the sinapine standard. The absorbance

**Figure 2.** Effect of the CM-Sephadex C25 ion-exchange column on purification of sinapine from rapeseed meal: spectrum of a crude methanol extract of Midas-1 meal; spectrum of the aqueous methanol effluent from a 0.6-mL CM-Sephadex C25 column; and spectrum of 2 M acetic acid/methanol eluate from the column.**Table 3. Absorbance of a Crude Methanol Extract, an Effluent, and an Eluate of 2 M Acetic Acid/Methanol (1:1, v/v) from a 0.6-mL CM-Sephadex C25 Ion-Exchange Column**

sample	peak wavelength (nm)	absorbance at peak ^a
crude extract ^b	238	1.797 ± 0.034
	330	
effluent ^c	245	0.942 ± 0.007
	326	1.264 ± 0.039
eluate ^b	230	0.823 ± 0.021
	245	0.286 ± 0.024
	330	0.505 ± 0.037
		0.593 ± 0.022

^a Mean of three replicates ± SD. ^b 0.5 mg of meal/mL. ^c 0.8 mg of meal/mL.

of the eluate at 330 nm was substantially lower than that of the original methanol extract (Table 3). The pooled effluent and water wash from the CM-Sephadex C25 column had a spectrum with peaks at 245 and 326 nm. The difference between meal extract and eluate was attributed to the effluent, which contained interfering compounds, such as other sinapyl esters and hydroxycinnamates (Strack, 1977), suggesting that the presence of these UV-absorbing compounds in the meal extract could interfere with sinapine determination if not removed. The HPLC analysis of eluate from CM-

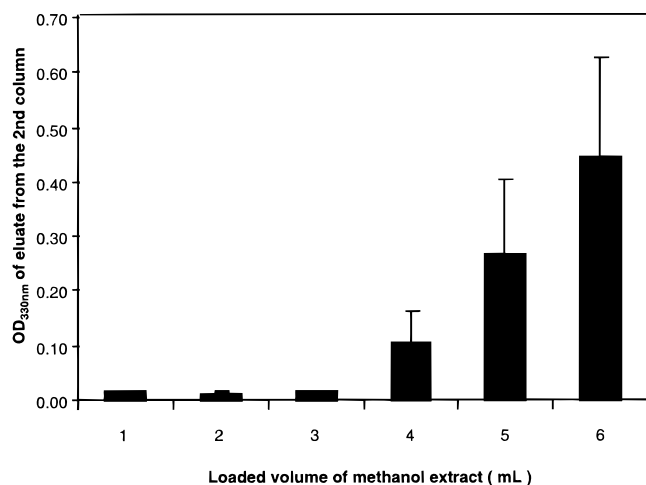


Figure 3. Capacity test of ion-exchange column. To a pair of 0.6-mL CM-Sephadex C25 columns was loaded 1–6 mL of methanol extract of Midas-1 meal (5 mg of meal/mL). The eluate of 10 mL of 2 M acetic acid/methanol (1:1 v/v) from the second of the pair was collected after washing with water, and the absorbance of the eluate was measured at 330 nm. The data presented are the mean of four replicates, and the error bars indicate standard deviation of the mean.

Sephadex C25 column showed that those non-sinapine compounds had been removed (data not shown). This result indicated that the use of an ion-exchange column increased the accuracy of the UV detection method.

Capacity of Ion-Exchange Column. The capacity of the CM-Sephadex C25 column for exchanging phenolic choline esters in meal extracts was measured by detecting excess sinapine in the second of a pair of ion-exchange columns. The absorbance at 330 nm of the eluate from the second column did not exceed 0.05 when ≤ 3 mL of Midas-1 meal extract (5 mg of meal/mL, equivalent to 15 mg of meal) were passed through the column (Figure 3). However, when ≥ 4 mL (equivalent to 20 mg of meal) of Midas-1 meal extract was applied to the column, the $A_{330\text{nm}}$ of eluate from the second column increased considerably (>0.1 OD unit), indicating that the capacity of the initial column had been exceeded. The maximum loading volume to a column packed with 0.6 mL of wet gel was 3–4 mL of crude extract from rapeseed meal (50 mg in 10 mL). To prevent the loss of sinapine during the purification procedure, the extract volume should not exceed 3 mL for a 0.6-mL CM-Sephadex C25 column. The used CM-Sephadex C25 column can be regenerated by treating with 3 mL of 1 N HCl followed by washing with 10 mL of water.

Elution Volume. With the addition of 1 or 2 mL (5 mg of meal/mL) of Midas-1 meal extract to a 0.6-mL CM-Sephadex C25 column, 10 mL of 2 M acetic acid/methanol was required for complete elution of phenolic choline esters ($>99\%$) (Figure 4). Most of the esters eluted in the fourth to ninth milliliter. Although the 11th mL of eluate still showed some absorbance at 330 nm, the first 10 mL contained $>99\%$ of the total loaded sinapine in the extract.

Reproducibility of the Ion-Exchange Column. The sinapine contents in Midas-1 meal extracts purified from eight ion-exchange columns ranged from 20.1 to 25.0 mg/g (mean = 22.4 ± 1.8 mg/g). The CV of sinapine measurements was 8.0% but was reduced to 3.3% when the extract was repeatedly purified from the same column. This indicates that the heterogeneity of the ion-

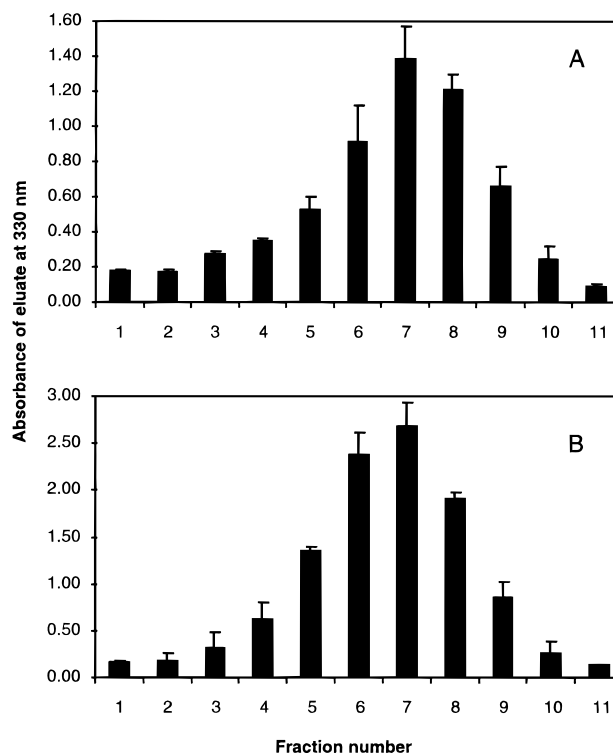


Figure 4. Test of minimum volume requirement of elution solvent. To a 0.6 mL CM-Sephadex C25 ion-exchange column was loaded (A) 1 mL or (B) 2 mL of methanol extract of Midas-1 meal (5 mg of meal/mL). The column was washed with water and eluted with 11×1 mL aliquots of 2 M acetic acid/methanol (1:1 v/v). Each elution fraction was collected, and its absorbance at 330 nm was measured. The data presented are the mean of three replicates, and error bars indicate standard deviation of mean.

Table 4. Recovery of Sinapine by IE-UV Spectrophotometric Method^a

sample	sinapine content ^b (μg)		recovery (%)
	without addition of sinapine isolate	with addition of sinapine isolate	
R-500	529 ± 42	584 ± 34	95.3
Midas-1	899 ± 54	1101 ± 56	113.6

^a Sinapine contents of R-500 and Midas-1 meals (50 mg) was determined as described under Materials and Methods. ^b Mean of five replicates \pm SD. ^c 80 μg of sinapine bisulfate isolated from *S. alba*.

exchange columns and the handling of elution procedure may increase the variability of assays.

Precision and Recovery. The overall mean of sinapine concentration in 80 meal samples was 18.3 mg/g with a pooled standard deviation of 0.7 mg/g. The CV was 3.9%. Sinapine recovery ranged from 95.3% to 113.6% when two levels of sinapine-containing meals were analyzed (Table 4). These results suggest that this method is reasonably precise for the routine analysis of sinapine content in rapeseed meal.

CONCLUSION

The 0.6-mL CM-Sephadex C25 column has the capacity to purify phenolic choline esters in ~ 15 mg of oil-extracted rapeseed meal. By using the IE-UV method combined with single-extraction and ion-exchange purification procedures, the sinapine content of rapeseed meal can be precisely measured in ~ 2.5 h with a CV of 3.9%. The linear range of sinapine concentration in the

sample for the measurement was from 0 to 20 $\mu\text{g/mL}$. Using this method, ~ 120 samples can be quantitatively analyzed by one person per day. This IE-UV method would overcome the speed limitation of HPLC method and has a broader range of applications in plant breeding, food/feed industries, and nutrition studies.

LITERATURE CITED

- Andersen, P. E. Double low rapeseed meal in diets to dairy cows. In *Advances in the Production and Utilization of Cruciferous Crops. World Crops: Production, Utilization, Description*; Sorensen, H., Ed.; Martinus Nijhoff: The Hague, 1985; pp 218–221.
- Andersen, H. R.; Sorensen, H. Double low rapeseed meal in diets to young bulls. In *Advances in the Production and Utilization of Cruciferous Crops. World Crops: Production, Utilization, Description*; Sorensen, H., Ed.; Martinus Nijhoff: The Hague, 1985; pp 208–217.
- Austin, F. L.; Wolff, I. A. Sinapine and related esters in seed meal of *Crambe abyssinica*. *J. Agric. Food Chem.* **1968**, *16*, 132–135.
- Bjerg, B.; Olsen, O.; Rasmussen, K. W.; Sorensen, H. New principles of ion-exchange techniques suitable to sample preparation and group separation of natural products prior to liquid chromatography. *J. Liq. Chromatogr.* **1984**, *7*, 691–707.
- Bouchereau, A.; Hamelin, J.; Lamour, I.; Renard, M.; Larher, F. Distribution of sinapine and related compounds in seeds of *Brassica* and allied genera. *Phytochemistry* **1991**, *30*, 1873–1881.
- Clandinin, D. R. Rapeseed oil meal studies. 4. Effect of sinapine, the bitter substance in rapeseed oil meal, on the growth of chickens. *Poult. Sci. J.* **1961**, *40*, 484–487.
- Clausen, S.; Olsen, O.; Sorensen, H. 4-Hydroxybenzoylcholine: a natural product present in *Sinapis alba*. *Phytochemistry* **1982**, *21*, 917–922.
- Clausen, S.; Olsen, O.; Sorensen, H. Separation of aromatic choline esters by high-performance liquid chromatography. *J. Chromatogr.* **1983**, *260*, 193–199.
- Clausen, S.; Larsen, L. M.; Ploger, A.; Sorensen, H. Aromatic choline esters in rapeseed. In *Advances in the Production and Utilization of Cruciferous Crops. World Crops: Production, Utilization, Description*; Sorensen, H., Ed.; Martinus Nijhoff: The Hague, 1985; pp 61–72.
- Henning, W. Rapid analysis of sinapine from mustard and mustard oil seeds by paired-ion high performance liquid chromatography. *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 345–348.
- Ismail, F.; Eskin, N. A. M. A new quantitative procedure for determination of sinapine. *J. Agric. Food Chem.* **1979**, *27*, 917–918.
- Ismail, F.; Vaisey-Genser, M.; Fyfe, B. Bitterness and astringency of sinapine and its components. *J. Food Sci.* **1981**, *46*, 1241–1244.
- Krygier, K.; Sosulski, F.; Hogge, L. Free, esterified, and insoluble-bound phenolic acids. 2. Composition of phenolic acids in rapeseed flour and hulls. *J. Agric. Food Chem.* **1982**, *30*, 334–336.
- Legueut, C.; Hocquemiller, R.; Cave, A. Quantitative procedure for determination of sinapine. *Ann. Pharm. Fr.* **1981**, *39*, 557–561.
- Raney, J. P.; Love, H. K.; Rakow, G. F. W.; Downey, R. K. An apparatus for rapid preparation of oil and oil-free meal from *Brassica* seed. *Fat Sci. Technol.* **1987**, *6*, 235–237.
- Strack, D. Sinapic acid ester fluctuations in cotyledons of *Raphanus sativus*. *Pflanzenphysiol.* **1977**, *84*, 139–145.
- Strack, D. Enzymatic synthesis of 1-sinapoylglucose from free sinapic acid and UDP-glucose by cell-free system from *Raphanus sativus* seedlings. *Z. Naturforsch.* **1980**, *35C*, 204–208.
- Thies, W. Determination of the phytic acid and sinapic acid esters in seeds of rapeseed and selection of genotypes with reduced concentrations of these compounds. *Fat Sci. Technol.* **1991**, *93*, 49–52.
- Tzagoloff, A. Metabolism of sinapine in mustard plant. I. Degradation of sinapine into sinapic acid and choline. *Plant Physiol.* **1963**, *38*, 202–206.

Received for review June 27, 1997. Revised manuscript received November 10, 1997. Accepted November 16, 1997. The financial support of the International Development Research Centre and the Canola Council of Canada for this project is gratefully acknowledged. Pacific Agri-Food Research Centre Contribution 1037.

JF9705577