

HPLC Analysis of the Anti-Nutrient, Sinapine, in Canola Seed

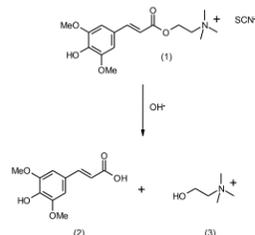
Keith J. Duff; Keith Richard, Hui Zhao
 EPL Bio Analytical Services, Inc., Niantic, IL
 www.eplbas.com • (217) 963-2143

Introduction

Most analytical methods for the analysis of sinapine (1) indirectly measure this anti-nutrient by first hydrolyzing the compound to sinapic acid (2) (with choline (3) as the by-product), giving total sinapic acid (Scheme 1). Endogenous sinapic acid is then analyzed without hydrolysis, and so sinapine content can be calculated by subtracting endogenous sinapic acid from the total sinapic acid. However, sinapic acid may also exist in nature in other forms, such as the glucosidic ester, glucopyranosyl sinapate¹. A more accurate strategy would be to analyze sinapine directly, which typically was not pursued, according to the literature, due to the lack of commercial availability of the sinapine standard².

This improvement would decrease analysis time by eliminating the hydrolysis step that may take from 30 minutes to two hours to complete³. Sinapine has previously been directly measured by HPLC, but the chromatographic conditions involved the use of an undesirable ion-pairing reagent and a rather complex mobile phase⁴. This work describes a simple, fast extraction and HPLC method for the direct measurement of sinapine in canola seed and canola processed fractions using a standard reversed phase column and mobile phase, and without the need for sample clean-up.

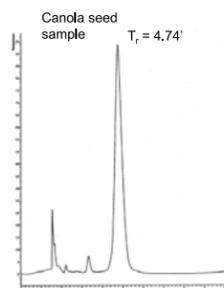
Scheme 1. Sinapine Thiocyanate Conversion to Sinapic Acid



Procedure

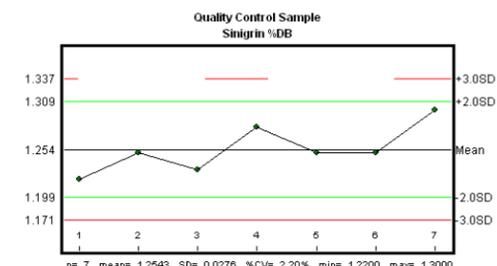
Spiked samples were fortified (~1X) with sinapine thiocyanate reference substance (EPL Bio Analytical Services, Niantic, IL). Canola seed or meal (unhulled or dehulled) was defatted with petroleum ether and extracted with DMF at 100°C for 5 minutes (x4). The combined extracts were diluted to volume (DMF) and filtered through a 0.45µm PTFE filter. The filtrate was directly injected onto a 150 x 4.6mm, 5µm, EagleEye™ C18 (AmChemteq, Inc., State College, PA) column at 30°C. The isocratic mobile phase of 85:15 – aqueous HOAc, pH 2.6: ACN was run at a flow rate of 1.0mL/min and UV detection at 330nm. Samples were quantified via a sinapine thiocyanate calibration curve plotted using Atlas v 8.2 software (Thermo-Fisher, Waltham, MA).

Results – Typical Sinapine Chromatogram



- Fast run time (6 min.)
- Relatively clean sample with little sample prep
- “Strong” DMF solvent doesn’t significantly impair chromatogram
- Conventional C18 column
- Simple, isocratic mobile phase

Reproducibility



Sinapine GLP Method Validation

Good reproducibility and accuracy for all three matrices:

Matrix	Sinapine Content (%DB: Avg. 7 Rep's)	% CV (Precision)	% DB Spike Recovery (Avg. 3 Rep's)
Canola Seed	1.25	2.07	83.6
Canola Dehulled Meal	1.41	1.23	90.6
Canola Unhulled Meal	1.32	2.66	104

Approximate: LOD: 100 ng/mL
 LOQ: 340 ng/mL

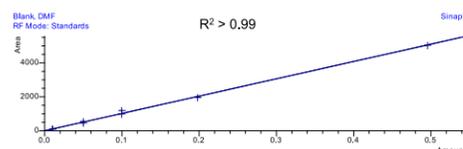
Discussion & Conclusions

- A simple, fast, and convenient method for analyzing sinapine was developed.
- The method was validated under GLP conditions for canola seed, unhulled toasted canola meal, and dehulled toasted canola meal.
- Excellent reproducibility and recovery were obtained.
- Method is probably amenable to running sinapic acid in the same run.
- Commercially available sinapine thiocyanate is the same salt form that occurs in nature; therefore, the extraction of the fortified samples closely mimics the natural sinapine extraction.

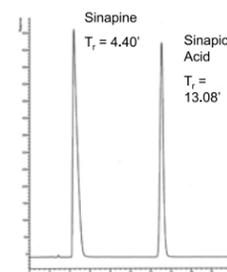
References

1. Amarowicz, R., Shahidi, F., "Chromatographic Separation of Glucopyranosyl Sinapate from Canola Meal," J. Am. Oil Chem. Soc., 1994, 71, 551-552.
2. Vuorela, S., Meyer, A.S., Heinonen, M., "Impact of Isolation Method on the Antioxidant Activity of Rapeseed meal Phenolics," Agric. Food Chem., 2004, 52, 8202-8207.
3. Li, J., El Rassi, Z., "High Performance Liquid Chromatography of Phenolic Choline Ester Fragments Derived by Chemical and Enzymatic Fragmentation Processes: Analysis of Sinapine in Rape Seed," J. Agric. Food Chem., 2002, 50, 1368-1373.
4. Matthaus, B., "Antioxidant Activity of Extracts Obtained from Residues of Different Oilseeds," J. Agric. Food Chem., 2002, 50, 3444-3452.

Typical Sinapine Calibration Curve



Simultaneous Sinapic Acid Analysis



- Injection of standards shown
- Sinapic acid (2) elutes with same isocratic mobile phase & conditions as used for sinapine (1)