

Application Brief 126

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Separation of Sinapine Thiocyanate in Semen Raphani Using an Acclaim Phenyl-1 Column

Semen Raphani (Figure 1), the seed of *Raphanus sativus L.*, is a Chinese medicinal plant commonly used for treatment of dyspepsia and hypertension. Its antihypertension properties are attributed to the presence of sinapine thiocyanate. The Chinese Pharmacopoeia (CP) monitors the quality control of Semen Raphani with a reversed-phase (RP) high-performance liquid chromatography (HPLC) method for the determination of sinapine thiocyanate (structure shown in Figure 2). The method specifies a stationary phase with phenyl groups bonded to silica.

This work describes a separation of sinapine thiocyanate using an Acclaim® Phenyl-1 column, which is based on covalent modification of high-purity, spherical, and porous silica particles with a specially designed silane ligand bearing a proprietary alkyl aromatic functionality.³ Figure 2 shows the separation of sinapine thiocyanate extracted from Semen Raphani following the CP method. The analyte of interest eluted as a symmetrical peak with adequate retention. The UV spectra of sinapine thiocyanate collected in the standard, Semen Raphani sample, and spiked Semen Raphani sample are highly consistent. The purity of sinapine thiocyanate may be estimated using the peak purity match factor, which can be calculated by Chromeleon® Chromatography Data System (CDS) software.

The calculated peak purity match factor for sinapine thiocyanate separated from the Semen Raphani extract is 950 (the corresponding value for 100% purity is 1000). These results demonstrate that the Acclaim Phenyl-1 column provides good selectivity and is suitable for analysis of sinapine thiocyanate.



Figure 1. Semen Raphani.

Column: Acclaim Phenyl-1 $(4.6 \times 150 \text{ mm}, 3 \mu\text{m})$ Column Temp.: 30 °C Acetonitrile/3% Acetic acid, 10/90 (v/v) Mobile Phase: Flow Rate: 1.5 mL/min Injection Volume: 5 μL UV, 326 nm Detection: Chromatograms: A. Mobile phase B. Sinapine thiocyanate standard (10 μg/mL) C. Semen Raphani sample D. Semen Raphani sample spiked with sinapine thiocyanate (5 $\mu g/mL$) Sample Preparation: Ultrasonic extraction in 70% methanol solution for 30 min 200--10 mAU 190 Sinapine Thiocyanate 80 328 D 0-224 C 190 80 329.0 -150 % 2 225 Minutes 400 190 28608

Figure 2. Chromatograms of sinapine thiocyanate on the Acclaim Phenyl-1 column following the CP method.

EQUIPMENT

Dionex UltiMate® 3000 RSLC system, including:

LPG 3400RS pump

WPS 3000RS autosampler

TCC 3000RS thermostatted column compartment

DAD 3000RS UV-vis detector

Chromeleon 6.80 SR9 software

REFERENCES

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- 2. Chinese Pharmacopoeia (Vol. 1), 2010, p 255.
- 3. Dionex Corporation, *Acclaim Phenyl-1 Unique Reversed-Phase Column with High Aromatic Selectivity*, Sunnyvale, CA. http://www.dionex.com/en-us/products/columns/lc/reversed-phase/acclaim-phenyl/lp-87227.html (accessed April 4, 2011).

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