**Sinapis Semen**

**Figure 1 (i)** A photograph of dried ripe seed of *Sinapis alba* L.

A. Seeds  B. Magnified image of seed  C. Magnified image of cut surface of seed

**Figure 1 (ii)** A photograph of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss.

A. Seeds  B. Magnified image of seed  C. Magnified image of cut surface of seed
1. NAMES

Official Name: Sinapis Semen

Chinese Name: 芥子

Chinese Phonetic Name: Jiezi

2. SOURCE

Sinapis Semen is the dried ripe seed of *Sinapis alba* L. or *Brassica juncea* (L.) Czern. et Coss. (Brassicaceae). The former is known as “Bai Jiezi”. The latter is known as “Huang Jiezi”. The plant is collected in late summer and early autumn when the fruit is ripe, afterwards the harvested plant is dried under the sun; the seeds tapped out, foreign matter removed, then the seeds are gathered to obtain Sinapis Semen.

**Part I  Dried ripe seed of *Sinapis alba* L.**

3. DESCRIPTION

Spheroidal, 1.5-2.6 mm in diameter.Externally greyish-white to pale yellow, finely reticulated, with an obvious dot-like hilum. Testa thin and brittle; after sectioning, pale yellow folded cotyledons visible, oily. Odour slight; taste pungent [Fig. 1 (i)].

4. IDENTIFICATION

4.1 Microscopic Identification (*Appendix III*)

**Transverse section**

Epidermal cells of testa subsquare or slightly radially elongated, containing mucilage with mucilaginous striations. Hypodermis consists of 2 layers of collenchymatous cells. Palisade cells of testa consist of 1 layer of cells with thickened inner and lateral walls and thin outer walls. Endosperm consists of 1 layer of subsquare cells, containing fatty oil droplets (before staining) and aleurone grains. Parenchymatous cells of radicle and cotyledons contain fatty oil droplets (before staining) and aleurone grains (Fig. 2).
Powder

Colour yellow. Epidermal cells of testa colourless, subrounded, subsquare, polygonal or subpolygonal in surface view, cellulose column visible at centre in some cells, mucilage striations visible in periphery. Palisade cells of testa with thickened inner and lateral walls and thin outer walls in lateral view, polygonal to slightly elongated-polygonal in surface view, 6-14 μm in diameter, walls thickened. Endosperm cells polygonal or rectangular in surface view, oblate to rectangular in lateral view, containing fatty oil droplets and aleurone grains. Cotyledon cells contain fatty oil droplets and aleurone grains (Fig. 3).
Figure 2  Microscopic features of transverse section of dried ripe seed of *Sinapis alba* L.

A. Sketch     B. Section illustration     C. Epidermal cells containing mucilage (mucilaginous striations )

5. Radicle  6. Cotyledon
Figure 3  Microscopic features of powder of dried ripe seed of *Sinapis alba* L. (under the light microscope)

1. Epidermal cells of testa in surface view
2. Palisade cells of testa (2-1 in lateral view, 2-2 in surface view)
3. Endosperm cells (3-1 in surface view, 3-2 in lateral view)  
4. Cotyledon cells
4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

**Standard solution**

*Sinapine thiocyanate standard solution*

Weigh 1.0 mg of sinapine thiocyanate CRS (Fig. 4) and dissolve in 1 mL of ethanol (50%).

**Developing solvent system**

Prepare a mixture of acetone, ethyl acetate, formic acid and water (5:3.5:1:0.5, v/v).

**Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (50%). Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (50%).

**Procedure**

Carry out the method by using a HPTLC silica gel F$_{254}$ plate and a freshly prepared developing solvent system as described above. Apply separately sinapine thiocyanate standard solution and the test solution (2 μL each) to the plate. Develop over a path of about 7 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the $R_f$ value by using the equation as indicated in Appendix IV (A).

![Chemical structure of sinapine thiocyanate](image)

**Figure 4** Chemical structure of sinapine thiocyanate
Figure 5  A reference HPTLC chromatogram of dried ripe seed of *Sinapis alba* L. extract observed under UV light (254 nm)

1. Sinapine thiocyanate standard solution   2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the $R_f$ value, corresponding to that of sinapine (Fig. 5).

### 4.3 High-Performance Liquid Chromatographic Fingerprinting *(Appendix XII)*

**Standard solution**

*Sinapine thiocyanate standard solution for fingerprinting, Std-FP (200 mg/L)*

Weigh 2.0 mg of sinapine thiocyanate CRS and dissolve in 10 mL of ethanol (50%).

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × g for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.
Chromatographic system
The liquid chromatograph is equipped with a DAD (225 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min.
Programme the chromatographic system as follows (Table 1) –

Table 1  Chromatographic system conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.1% Trifluoroacetic acid (% v/v)</th>
<th>Acetonitrile (% v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>95 → 30</td>
<td>5 → 70</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

System suitability requirements
Perform at least five replicate injections, each using 10 µL of sinapine thiocyanate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of sinapine should not be more than 5.0%; the RSD of the retention time of sinapine peak should not be more than 2.0%; the column efficiency determined from sinapine peak should not be less than 43000 theoretical plates.

The R value between peak 2 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 6).

Procedure
Separately inject sinapine thiocyanate Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of sinapine peak in the chromatogram of sinapine thiocyanate Std-FP and the retention times of the six characteristic peaks (Fig. 6) in the chromatogram of the test solution. Identify sinapine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sinapine thiocyanate Std-FP. The retention times of sinapine peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the six characteristic peaks of dried ripe seed of Sinapis alba L. extract are listed in Table 2.
Table 2  The RRTs and acceptable ranges of the six characteristic peaks of dried ripe seed of *Sinapis alba* L. extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.68</td>
<td>± 0.03</td>
</tr>
<tr>
<td>2 (marker, sinapine)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>1.07</td>
<td>± 0.03</td>
</tr>
<tr>
<td>4</td>
<td>1.14</td>
<td>± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>1.39</td>
<td>± 0.03</td>
</tr>
<tr>
<td>6</td>
<td>1.87</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>

Figure 6  A reference fingerprint chromatogram of dried ripe seed of *Sinapis alba* L. extract

For positive identification, the sample must give the above six characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 6).
5. TESTS

5.1 Heavy Metals (*Appendix V*): meet the requirements.

5.2 Pesticide Residues (*Appendix VI*): meet the requirements.

5.3 Mycotoxins (*Appendix VII*): meet the requirements.

5.4 Sulphur Dioxide Residues (*Appendix XVI*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 1.0%.

5.6 Ash (*Appendix IX*)

   Total ash: not more than 5.0%.
   Acid-insoluble ash: not more than 0.5%.

5.7 Water Content (*Appendix X*)

   Oven dried method: not more than 9.0%.

6. EXTRACTIVES (*Appendix XI*)

   Water-soluble extractives (cold extraction method): not less than 20.0%.
   Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. ASSAY

   Carry out the method as directed in Appendix IV (B).

   **Standard solution**
   
   *Sinapine thiocyanate standard stock solution, Std-Stock (500 mg/L)*
   
   Weigh accurately 5.0 mg of sinapine thiocyanate CRS and dissolve in 10 mL of ethanol (50%).

   *Sinapine thiocyanate standard solution for assay, Std-AS*
   
   Measure accurately the volume of the sinapine thiocyanate Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 12.5, 50, 100, 150, 250 mg/L for sinapine thiocyanate.

   **Test solution**
   
   Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × g for 5 min. Filter
and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (329 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 3) –

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.1% Trifluoroacetic acid (% v/v)</th>
<th>Acetonitrile (% v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>95 → 30</td>
<td>5 → 70</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

**System suitability requirements**

Perform at least five replicate injections, each using 10 µL of sinapine thiocyanate Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of sinapine should not be more than 5.0%; the RSD of the retention time of sinapine peak should not be more than 2.0%; the column efficiency determined from sinapine peak should not be less than 43000 theoretical plates.

The $R$ value between sinapine peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 7).

**Calibration curve**

Inject a series of sinapine thiocyanate Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of sinapine against the corresponding concentrations of sinapine thiocyanate Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

**Procedure**

Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify sinapine peak (Fig. 7) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sinapine thiocyanate Std-AS. The retention times of sinapine peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of sinapine thiocyanate in the test solution, and calculate the percentage content of sinapine (the percentage content of sinapine thiocyanate × 0.84, where 0.84 is the molar mass ratio of sinapine and sinapine thiocyanate) in the sample by using the equations as indicated in Appendix IV (B).
Limits

The dried ripe seed of *Sinapis alba* L. contains not less than 0.57% of sinapine (C_{16}H_{24}NO_{5}), calculated with reference to the dried substance.

![Figure 7](image)

**Figure 7** A reference assay chromatogram of dried ripe seed of *Sinapis alba* L. extract
Part II  Dried ripe seed of *Brassica juncea* (L.) Czern. et Coss.

3. DESCRIPTION

Spheroidal, relatively small, 1-2 mm in diameter. Externally yellow to brownish-yellow, a few dark reddish-brown. Testa thin and brittle; after sectioning, pale yellow folded cotyledons visible, oily. Odour slight, characteristic and pungent after tritivated and moistened with water [Fig. 1 (ii)].

4. IDENTIFICATION

4.1 Microscopic Identification (*Appendix III*)

**Transverse section**

Epidermal cells of testa tangentially elongated, containing mucilage, mucilaginous striations indistinct. Hypodermis consists of 1 layer of thin-walled cells. Palisade cells of testa consist of 1 layer of cells with thickened inner and lateral walls and thin outer walls. Endosperm consists of 1 layer of subsquare cells, containing fatty oil droplets (before staining) and aleurone grains. Parenchymatous cells of radicle and cotyledons contain fatty oil droplets (before staining) and aleurone grains (Fig. 8).

**Powder**

Colour yellow. Epidermal cells of testa colourless, polygonal or subpolygonal in surface view, mucilage striations indistinct. Palisade cells of testa with thickened inner and lateral walls and thin outer walls in lateral view, polygonal to slightly elongated-polygonal in surface view, 7-17 μm in diameter, walls thickened. Endosperm cells polygonal or rectangular in surface view, oblate to rectangular in lateral view, containing fatty oil droplets and aleurone grains. Cotyledon cells contain fatty oil droplets and aleurone grains (Fig. 9).
**Figure 8** Microscopic features of transverse section of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss.

A. Sketch  
B. Section illustration  
C. Epidermal cells containing mucilage (indistinct mucilaginous striations →)

1. Epidermis of testa  
2. Hypodermis  
3. Palisade cells of testa  
4. Endosperm  
5. Radicle  
6. Cotyledon
Figure 9  Microscopic features of powder of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss.
(under the light microscope)

1. Epidermal cells of testa in surface view
2. Palisade cells of testa (2-1 in lateral view, 2-2 in surface view)
3. Endosperm cells (3-1 in surface view, 3-2 in lateral view)  4. Cotyledon cells
4.2 Thin-Layer Chromatographic Identification [Appendix IV(A)]

**Standard solution**

*Sinapine thiocyanate standard solution*

Weigh 1.0 mg of sinapine thiocyanate CRS (Fig. 10) and dissolve in 1 mL of ethanol (50%).

**Developing solvent system**

Prepare a mixture of acetone, ethyl acetate, formic acid and water (5:3.5:1:0.5, v/v).

**Test solution**

Weigh 0.2 g of the powdered sample and place it in a 50-mL conical flask, then add 10 mL of ethanol (50%). Sonicate (270 W) the mixture for 30 min. Filter and transfer the filtrate to a 50-mL round-bottomed flask. Evaporate the solvent to dryness at reduced pressure in a rotary evaporator. Dissolve the residue in 1 mL of ethanol (50%).

**Procedure**

Carry out the method by using a HPTLC silica gel F$_{254}$ plate and a freshly prepared developing solvent system as described above. Apply separately sinapine thiocyanate standard solution and the test solution (2 μL each) to the plate. Develop over a path of about 7 cm. After the development, remove the plate from the chamber, mark the solvent front and dry in air. Examine the plate under UV light (254 nm). Calculate the $R_f$ value by using the equation as indicated in Appendix IV (A).

![Chemical structure of sinapine thiocyanate](image)

**Figure 10** Chemical structure of sinapine thiocyanate
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Figure 11  A reference HPTLC chromatogram of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss. extract observed under UV light (254 nm)

1. Sinapine thiocyanate standard solution  2. Test solution

For positive identification, the sample must give spot or band with chromatographic characteristics, including the colour and the $R_f$ value, corresponding to that of sinapine (Fig. 11).

4.3 High-Performance Liquid Chromatographic Fingerprinting (*Appendix XII*)

**Standard solution**

*Sinapine thiocyanate standard solution for fingerprinting, Std-FP (200 mg/L)*

Weigh 2.0 mg of sinapine thiocyanate CRS and dissolve in 10 mL of ethanol (50%).

**Test solution**

Weigh 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about $5000 \times g$ for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.
Chromatographic system
The liquid chromatograph is equipped with a DAD (329 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 µm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 4) –

Table 4   Chromatographic system conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.1% Trifluoroacetic acid (%, v/v)</th>
<th>Acetonitrile (%, v/v)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>95 → 30</td>
<td>5 → 70</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

System suitability requirements
Perform at least five replicate injections, each using 10 µL of sinapine thiocyanate Std-FP. The requirements of the system suitability parameters are as follows: the RSD of the peak area of sinapine should not be more than 5.0%; the RSD of the retention time of sinapine peak should not be more than 2.0%; the column efficiency determined from sinapine peak should not be less than 43000 theoretical plates.

The \( R \) value between peak 3 and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 12).

Procedure
Separately inject sinapine thiocyanate Std-FP and the test solution (10 µL each) into the HPLC system and record the chromatograms. Measure the retention time of sinapine peak in the chromatogram of sinapine thiocyanate Std-FP and the retention times of the five characteristic peaks (Fig. 12) in the chromatogram of the test solution. Identify sinapine peak in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sinapine thiocyanate Std-FP. The retention times of sinapine peaks from the two chromatograms should not differ by more than 2.0%. Calculate the RRTs of the characteristic peaks by using the equation as indicated in Appendix XII.

The RRTs and acceptable ranges of the five characteristic peaks of dried ripe seed of \( Brassica juncea \) (L.) Czern. et Coss. extract are listed in Table 5.
Table 5  The RRTs and acceptable ranges of the five characteristic peaks of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss. extract

<table>
<thead>
<tr>
<th>Peak No.</th>
<th>RRT</th>
<th>Acceptable Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.82</td>
<td>± 0.03</td>
</tr>
<tr>
<td>2</td>
<td>0.98</td>
<td>± 0.03</td>
</tr>
<tr>
<td>3 (marker, sinapine)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>1.06</td>
<td>± 0.03</td>
</tr>
<tr>
<td>5</td>
<td>1.16</td>
<td>± 0.03</td>
</tr>
</tbody>
</table>

Figure 12  A reference fingerprint chromatogram of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss. extract

For positive identification, the sample must give the above five characteristic peaks with RRTs falling within the acceptable range of the corresponding peaks in the reference fingerprint chromatogram (Fig. 12).

5. TESTS

5.1 Heavy Metals (*Appendix V*): meet the requirements.

5.2 Pesticide Residues (*Appendix VI*): meet the requirements.

5.3 Mycotoxins (*Appendix VII*): meet the requirements.

5.4 Sulphur Dioxide Residues (*Appendix XVI*): meet the requirements.

5.5 Foreign Matter (*Appendix VIII*): not more than 1.0%.
5.6 **Ash** *(Appendix IX)*

Total ash: not more than 4.5%.
Acid-insoluble ash: not more than 0.5%.

5.7 **Water Content** *(Appendix X)*

Oven dried method: not more than 8.0%.

6. **EXTRACTIVES** *(Appendix XI)*

Water-soluble extractives (cold extraction method): not less than 19.0%.
Ethanol-soluble extractives (cold extraction method): not less than 13.0%.

7. **ASSAY**

Carry out the method as directed in Appendix IV (B).

**Standard solution**

*Sinapine thiocyanate standard stock solution, Std-Stock (500 mg/L)*
Weigh accurately 5.0 mg of sinapine thiocyanate CRS and dissolve in 10 mL of ethanol (50%).

*Sinapine thiocyanate standard solution for assay, Std-AS*
Measure accurately the volume of the sinapine thiocyanate Std-Stock, dilute with ethanol (50%) to produce a series of solutions of 12.5, 50, 100, 150, 250 mg/L for sinapine thiocyanate.

**Test solution**

Weigh accurately 1.0 g of the powdered sample and place it in a 50-mL centrifuge tube, then add 25 mL of ethanol (50%). Sonicate (270 W) the mixture for 30 min. Centrifuge at about 5000 × g for 5 min. Filter and transfer the filtrate to a 50-mL volumetric flask. Repeat the extraction for one more time. Combine the filtrates and make up to the mark with ethanol (50%). Filter through a 0.45-μm RC filter.

**Chromatographic system**

The liquid chromatograph is equipped with a DAD (329 nm) and a column (4.6 × 250 mm) packed with ODS bonded silica gel (5 μm particle size). The flow rate is about 1.0 mL/min. Programme the chromatographic system as follows (Table 6) –
Table 6  Chromatographic system conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>0.1% Trifluoroacetic acid (%)</th>
<th>Acetonitrile (%)</th>
<th>Elution</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 – 30</td>
<td>95 → 30</td>
<td>5 → 70</td>
<td>linear gradient</td>
</tr>
</tbody>
</table>

System suitability requirements
Perform at least five replicate injections, each using 10 µL of sinapine thiocyanate Std-AS (100 mg/L). The requirements of the system suitability parameters are as follows: the RSD of the peak area of sinapine should not be more than 5.0%; the RSD of the retention time of sinapine peak should not be more than 2.0%; the column efficiency determined from sinapine peak should not be less than 43000 theoretical plates.

The $R$ value between sinapine peak and the closest peak in the chromatogram of the test solution should not be less than 1.5 (Fig. 13).

Calibration curve
Inject a series of sinapine thiocyanate Std-AS (10 µL each) into the HPLC system and record the chromatograms. Plot the peak areas of sinapine against the corresponding concentrations of sinapine thiocyanate Std-AS. Obtain the slope, y-intercept and the $r^2$ value from the 5-point calibration curve.

Procedure
Inject 10 µL of the test solution into the HPLC system and record the chromatogram. Identify sinapine peak (Fig. 13) in the chromatogram of the test solution by comparing its retention time with that in the chromatogram of sinapine thiocyanate Std-AS. The retention times of sinapine peaks from the two chromatograms should not differ by more than 5.0%. Measure the peak area and calculate the concentration (in milligram per litre) of sinapine thiocyanate in the test solution, and calculate the percentage content of sinapine (the percentage content of sinapine thiocyanate × 0.84, where 0.84 is the molar mass ratio of sinapine and sinapine thiocyanate) in the sample by using the equations as indicated in Appendix IV (B).

Limits
The dried ripe seed of *Brassica juncea* (L.) Czern. et Coss. contains not less than 0.42% of sinapine ($C_{16}H_{24}NO_5$), calculated with reference to the dried substance.
**Figure 13** A reference assay chromatogram of dried ripe seed of *Brassica juncea* (L.) Czern. et Coss. extract