There are countless chemicals used by the pharmaceutical industry to make drugs and biomedical media. The data in this booklet will help determine the proper analytical conditions for High Performance Liquid Chromatography (HPLC). Shimadzu will answer questions about compounds not included in the booklet; contact your Shimadzu representative for additional information.
# Pharmaceutical Application Data

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Common Pharmaceutical Components

Figure 1-1 Analysis of Allantoin in a Cream

Figure 1-2 Analysis of Piroctone

Analytical Conditions

<table>
<thead>
<tr>
<th>Compound</th>
<th>1. Allantoin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Asahipak ODP-50 4D (150mm × 4.6mm i.d.)</td>
</tr>
</tbody>
</table>
| Mobile Phase | A: Methanol containing 100mM 2-aminoethanol and 50mM perchloric acid  
B: Acetonitrile  
A / B = 2 / 8 (v/v) |
| Flow Rate | 0.6mL/min |
| Temperature | 25°C |
| Detection | Absorption (300nm) |

Sample Pretreatment

1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

Notes

The amino resin in the Asahipak NH2-P50 4E stationary phase is basic. Before analysis, flush the column with 100mM sodium phosphate buffer (pH 2.2) at a flow rate of 1.0mL/min for approximately 30 minutes to acidify the resin.

<table>
<thead>
<tr>
<th>Compound</th>
<th>1. Piroctone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Asahipak NH2-P50 4E (250mm × 4.6mm i.d.)</td>
</tr>
</tbody>
</table>
| Mobile Phase | A: 10mM sodium phosphate buffer (pH 2.6)  
B: Acetonitrile  
A / B = 2 / 8 (v/v) |
| Flow Rate | 1.0mL/min |
| Temperature | 40°C |
| Detection | Absorption (200nm) |

Sample Pretreatment

1. The sample was diluted with distilled water, then sonicated for 5 minutes.
2. The sample solution was heated for 5 minutes at 60°C.
3. The hot solution was vortexed, then membrane filtered (0.45µm).
4. After cooling, a 10µL aliquot of filtrate was injected.

Notes

Piroctone peaks are usually crooked because of interactions with the metal ions in the ODS stationary phase.
Figure 1-3  Analysis of Naphazoline and Chlorpheniramine in an Eyewash

### Analytical Conditions

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate  
  B: Acetonitrile  
  A / B = 2 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (254nm)

#### Sample Pretreatment

1. The sample was membrane filtered (0.45µm).
2. 5µL of filtrate were injected.

#### Compounds

1. Naphazoline
2. Chlorpheniramine

Figure 1-4  Analysis of Zinc Pyrithione in a Lotion

### Analytical Conditions

- **Column**: L-Column ODS (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium acetate buffer (pH 4.0) with 3mM ethylenediaminetetraacetic acid disodium salt  
  B: Acetonitrile  
  A / B = 10 / 1 (v/v)
- **Flow Rate**: 0.8mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (250nm)

#### Sample Pretreatment

1. The sample was diluted with Solution C (see below), then sonicated until dissolved.
2. The sample solution was membrane filtered (0.45µm).
3. A 10µL aliquot of filtrate was injected.

#### Notes

1. Preparation of Solution C [10mM sodium phosphate buffer (pH 11.0) with 2mM ethylenediaminetetraacetic acid disodium salt]  
   Combine 654.48mg of anhydrous ethylenediaminetetraacetic acid disodium salt, 1790.7mg of dibasic sodium phosphate (12H₂O and 1900.6mg of tribasic sodium phosphate (12H₂O, and bring up the volume to 1L with distilled water.
2. Preparation of 10mM Sodium Acetate Buffer (pH 4.0)  
   Combine 517µL of acetic acid (99.5%) and 82mg sodium acetate, and bring up the volume to 1L with distilled water.
**Analytical Conditions**

<table>
<thead>
<tr>
<th>Compound</th>
<th>1. Biosol</th>
</tr>
</thead>
</table>

**Column**
STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**
A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Acetonitrile
A / B = 2 / 3 (v/v)

**Flow Rate**
1.0mL/min

**Temperature**
40°C

**Detection**
Absorption (280nm)

**Sample Pretreatment**
1. The sample was diluted with mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. A 10µL aliquot of filtrate was injected.

---

**Analytical Conditions**

<table>
<thead>
<tr>
<th>Compound</th>
<th>1. Nalbuphine</th>
</tr>
</thead>
</table>

**Column**
STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**
A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Acetonitrile
A / B = 3 / 1 (v/v)

**Flow Rate**
1.0mL/min

**Temperature**
25°C

**Detection**
Absorption (210nm)

**Sample Pretreatment**
1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 50µL of filtrate were injected.
**Analytical Conditions**

Column: STR ODS-M (150mm × 4.6mm i.d.)

Mobile Phase:
- A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
- B: Acetonitrile
- A / B = 55 / 45 (v/v)

Flow Rate: 0.8mL/min

Temperature: 45°C

Detection: Absorption (325nm)

**Sample Pretreatment**

1. The sample was crushed, then dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 50µL of filtrate were injected.

---

**Analytical Conditions**

Column: STR ODS-II (150mm × 4.6mm i.d.)

Mobile Phase:
- A: 10mM sodium phosphate buffer (pH 2.6)
- B: Acetonitrile
- A / B = 6 / 4 (v/v)

Flow Rate: 0.8mL/min

Temperature: 40°C

Detection: Absorption (250nm)

**Sample Pretreatment**

1. The sample was dispersed in a small volume of tetrahydrofuran (HPLC grade).
2. The sample solution was diluted with 10mM dibasic sodium phosphate solution.
3. The sample solution was membrane filtered (0.45mm).
4. A 5µL aliquot of filtrate was injected.
**Analytical Conditions**

Column: STR ODS-II (150mm × 4.6mm i.d.)
Mobile Phase: A: 20mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate  
B: Acetonitrile  
A / B = 3 / 1 (v/v)  
Flow Rate: 1.0mL/min  
Temperature: 50°C  
Detection: Absorption (210nm)

**Sample Pretreatment**

1. The sample was dissolved in mobile phase.  
2. The sample solution was membrane filtered (0.45µm).  
3. 10µL of filtrate were injected.

---

**Analytical Conditions**

Column: STR ODS-M (150mm × 4.6mm i.d.)  
Mobile Phase: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate  
B: Methanol  
A / B = 4 / 1 (v/v)  
Flow Rate: 0.8mL/min  
Temperature: 50°C  
Detection: Absorption (210nm)

**Sample Pretreatment**

1. The sample (500mg) was dispersed in 20mL of 0.1N perchloric acid / methanol [1 / 1 (v/v)], then sonicated for five minutes.  
2. The sample solution was agitated in a 60°C water bath for 10 minutes.  
3. After cooling, the solution was membrane filtered (0.45µm).  
4. A 5µL aliquot of filtrate was injected.
**Analytical Conditions**

Column: STR ODS-M (150mm × 4.6mm i.d.)
Mobile Phase: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Methanol
A / B = 3 / 2 (v/v)
Flow Rate: 0.8mL/min
Temperature: 50°C
Detection: Absorption (210nm)

**Sample Pretreatment**

1. The sample (500mg) was dispersed in 20mL of 0.1N perchloric acid / methanol [1 / 1 (v/v)], then sonicated for 5 minutes.
2. The sample solution was agitated in a 60°C water bath for 10 minutes.
3. After cooling, the solution was membrane filtered (0.45µm).
4. A 5µL aliquot of filtrate was injected.

---

**Analytical Conditions**

Column: STR ODS-M (150mm × 4.6mm i.d.)
Mobile Phase: A: 10mM sodium phosphate buffer (pH 2.6)
B: Acetonitrile
A / B = 1 / 4 (v/v)
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: Absorption (275nm)
## Analytical Conditions

**Column**: STR ODS-M (150mm × 4.6mm i.d.)

**Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Acetonitrile
A / B = 850 / 15 (v/v)

**Flow Rate**: 0.8mL/min

**Temperature**: 40°C

**Detection**: Absorption (260nm)

### Sample Pretreatment

1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

---

## Analytical Conditions

**Column**: STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Acetonitrile
A / B = 1 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Fluorescence (Ex. 260nm, Em. 355nm)

### Sample Pretreatment

1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 20µL of filtrate were injected.
**Analytical Conditions**

**Column**: Asahipak NH2P-50 4E (250mm \( \times \) 4.6mm i.d.)

**Mobile Phase**:  
- A: 10mM sodium phosphate buffer (pH 7.0)  
- B: Acetonitrile  
  \[ \text{A} / \text{B} = 1 / 1 \ (v/v) \]

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (210nm)

**Sample Pretreatment**
1. 10mg of sample were dissolved in enough mobile phase to bring up the volume to 10mL.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

---

**Analytical Conditions**

**Column**: STR ODS-M (150mm \( \times \) 4.6mm i.d.)

**Mobile Phase**:  
- A: Water  
- B: Acetonitrile  
  \[ \text{A} / \text{B} = 1 / 1 \ (v/v) \]

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Refractive Index

**Sample Pretreatment**
1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.
Figure 1-17  Analysis of Anodynine

- **Analytical Conditions**
  - **Column**: STR ODS-II (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 20mM 1-heptanesulfonic acid sodium salt
    - B: Methanol
    - C: Acetonitrile
    - A / B / C = 3 / 2 / 2 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 45°C
  - **Detection**: Absorption (210nm)

- **Sample Pretreatment**
  1. The sample was diluted by a factor of 10 with ethanol.
  2. The sample solution was membrane filtered (0.45µm).
  3. A 5µL aliquot of filtrate was injected.

- **Compounds**
  1. Salicylic Acid
  2. Diphenhydramine
  3. Methyl Salicylate
  4. Camphor
  5. Thymol

Figure 1-18  Analysis of Undecylenic Acid

- **Analytical Conditions**
  - **Column**: STR ODS-M (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6)
    - B: Acetonitrile
    - A / B = 1 / 3 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 40°C
  - **Detection**: Absorption (210nm)

- **Sample Pretreatment**
  1. The sample was diluted by a factor of 10 with ethanol.
  2. The sample solution was membrane filtered (0.45µm).
  3. A 5µL aliquot of filtrate was injected.

- **Compound**
  1. Undecylenic Acid
**Figure 1-19  Analysis of Thianthol**

**Analytical Conditions**
- **Column**: STR ODS-M (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6)
  B: Acetonitrile
  A / B = 1 / 3 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (260nm)

**Sample Pretreatment**
1. The sample was diluted by a factor of 10 with ethanol.
2. The sample solution was membrane filtered (0.45µm).
3. A 5µL aliquot of filtrate was injected.

---

**Figure 1-20  Analysis of Ursodeoxycholic Acid**

**Analytical Conditions**
- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6)
  B: Acetonitrile
  A / B = 1 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (210nm)

**Sample Pretreatment**
1. The sample was dissolved in enough mobile phase to make a 1mg/L sample solution.
2. The solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.
### Analytical Conditions

**Column**: STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**
- A: 20mM phosphoric acid with 10mM triethylamine
- B: Acetonitrile
- A / B = 20 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 25°C

**Detection**: Absorption (240nm)

### Sample Pretreatment

1. Distilled water was added to 1mL of sample to bring up the volume to 100mL.
2. The sample solution was diluted by a factor of 500 with mobile phase.
3. The solution was membrane filtered (0.45µm).
4. 10µL of filtrate were injected.

---

### Analytical Conditions

**Column**: Shim-pack VP-ODS (150mm × 4.6mm i.d.)

**Mobile Phase**
- A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
- B: Acetonitrile
- A / B = 3 / 2 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (260nm)

### Sample Pretreatment

1. The sample was diluted in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. A 10µL aliquot of filtrate was injected.
## Analytical Conditions

**Column**  
STR ODS-II (150mm x 4.6mm i.d.)

**Mobile Phase**  
A: 10mM sodium phosphate buffer (pH 2.6) with 5mM sodium 1-octanesulfonate and 5mM 2-aminoethanol  
B: Methanol  
A / B = 10 / 1 (v/v)

**Flow Rate**  
1.0mL/min

**Temperature**  
50°C

**Detection (postcolumn derivatization method)**

**Reagents**  
A: 20mM sodium borate buffer (pH 9.2)  
B: 20mM α-phthalaldehyde methanolic solution  
A / B = 5 / 1 (v/v)

**Flow Rate**  
0.5mL/min

**Reaction coil**  
piping part J

**Temperature**  
50°C

**Detection**  
Fluorescence (Ex. 340nm, Em. 450nm)

## Sample Pretreatment

1. The sample tablets were added to 100mL of 10mM perchloric acid and shaken for approximately 30 minutes.
2. The sample solution was membrane filtered (0.45µm).
3. The filtrate was diluted by a factor of 100 with 10mM perchloric acid.
4. 10µL of dilute filtrate were injected.

## Analytical Conditions

**Column**  
STR ODS-II (150mm x 4.6mm i.d.)

**Mobile Phase**  
A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate  
B: Acetonitrile  
A / B = 1 / 4 (v/v)

**Flow Rate**  
1.0mL/min

**Temperature**  
40°C

**Detection**  
Absorption (210nm)

## Notes

Benzalkonium and its homologues with alkyl groups of varying length are eluted with the above analytical conditions.
### Analytical Conditions

**Column**: Asahipak ES-502C (100mm × 7.6mm i.d.)

**Mobile Phase**: A: 20mM sodium phosphate buffer (pH 6.9)
B: Acetonitrile
A / B = 1 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (210nm)

### Notes

Benzalkonium is eluted with its homologues under the above analytical conditions.

### Analytical Conditions

**Column**: Asahipak GS-220HQ (300mm × 7.6mm i.d.)

**Mobile Phase**: 5mM perchloric acid

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Refractive Index

### Sample Pretreatment

1. The sample was diluted by a factor of 10 with mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. A 10µL aliquot of filtrate was injected.
Figure 1-27 Analysis of Chondroitin Sulfate

- **Analytical Conditions**
  - **Column**: Asahipak GF-510HQ (300mm × 7.6mm i.d.)
  - **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate, B: Acetonitrile, A / B = 65 / 35 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 70°C
  - **Detection**: Absorption (210nm)

- **Sample Pretreatment**
  1. The sample was membrane filtered (0.45µm).
  2. 20µL of filtrate were injected.

Figure 1-28 Analysis of Ethynylestradiol

- **Analytical Conditions**
  - **Column**: STR ODS-II (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: 20mM sodium phosphate buffer (pH 2.5), B: Acetonitrile, A / B = 5 / 4 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 40°C
  - **Detection**: Fluorescence (Ex. 280nm, Em. 320nm)

- **Sample Pretreatment**
  1. The sample was membrane filtered (0.45µm).
  2. 10µL of filtrate were injected.

- **Notes**
  The column was flushed with a rinse solution [A / B=1 / 4 (v/v)] for two minutes, then conditioned with mobile phase for 10 minutes per analysis.
### Analytical Conditions

**Column**: Asahipak GS-220H (250mm × 7.6mm i.d.)

**Mobile Phase**: 10mM sodium phosphate buffer (pH 2.6)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (210nm)

### Sample Pretreatment

1. The sample was membrane filtered (0.45µm).
2. A 5µL aliquot of filtrate was injected.

---

### Analytical Conditions

**Column**: STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**: A: Methanol  
B: Ethanol  
A / B = 1 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (250nm)

### Sample Pretreatment

1. The sample was dispersed in 2mL of tetrahydrofuran and diluted with ethanol to bring up the volume to 10mL.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.
**Analytical Conditions**

- **Column**: STR ODS-II (250mm x 4.6mm i.d.)
- **Mobile Phase**: A: Methanol  
  B: Ethanol  
  A / B = 7 / 3 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 45°C
- **Detection**: Absorption (235nm)

**Sample Pretreatment**

1. The sample was dispersed in 2mL of tetrahydrofuran and diluted with ethanol to bring up the volume to 10mL.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

---

**Analytical Conditions**

- **Column**: Shim-pack ISA-07 (250mm x 4.0mm i.d.)
- **Mobile Phase**: 100mM sodium borate buffer (pH 9.2) with 50mM sodium perchlorate and 10mM arginine
- **Flow Rate**: 0.6mL/min
- **Temperature**: 80°C

**Detection (postcolumn derivatization method)**

- **Reaction coil**: SUS tubing (2.0m x 0.3mm i.d.)
- **Temperature**: 150°C
- **Cooling coil**: SUS tubing (2.0m x 0.3mm i.d.)
- **Detection**: Fluorescence (Ex. 320nm, Em. 430nm)
Analytical Conditions

Column: STR ODS-M (150mm × 4.6mm i.d.)
Mobile Phase:
- A: 10mM sodium phosphate buffer (pH 2.6) with 200mM sodium perchlorate
- B: Methanol
  A / B = 3 / 7 (v/v)
Flow Rate: 0.8mL/min
Temperature: 40°C
Detection: Absorption (260nm)

Sample Pretreatment
1. The sample was diluted with ethanol and shaken.
2. The sample solution was heated for 10 minutes at 50°C.
3. The solution was cooled and separated.
4. The clear upper layer of the solution was membrane filtered (0.45µm).
5. A 10µL aliquot of filtrate was injected.
Figure 1-35  Analysis of Resorcin, Swertiamarin and Cepharanthine in an Insect Repellent

**Compounds**
1. Resorcin
2. Swertiamarin
3. Cepharanthine
4. Light Sensitive Reagent No.301

**Analytical Conditions**
- **Column**: L-Column ODS (150mm x 4.6mm i.d.)
- **Mobile Phase**: Gradient elution
  - A: 10mM sodium phosphate buffer with 100mM sodium perchlorate / Acetonitrile = 10 / 1 (v/v)
  - B: 10mM sodium phosphate buffer with 100mM sodium perchlorate / Acetonitrile = 1 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (240nm up to 7.0min.; 240nm from 7.0-14.5min.; 370nm after 14.5min.)

**Gradient Protocol**

<table>
<thead>
<tr>
<th>Time (minutes)</th>
<th>Conc. of B (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>5</td>
</tr>
<tr>
<td>4.90</td>
<td>5</td>
</tr>
<tr>
<td>5.00</td>
<td>70</td>
</tr>
<tr>
<td>16.00</td>
<td>70</td>
</tr>
<tr>
<td>16.10</td>
<td>5</td>
</tr>
</tbody>
</table>

**Sample Pretreatment**
1. The sample was diluted with ethanol and shaken.
2. The sample solution was heated for 10 minutes at 50°C.
3. The solution was cooled and separated.
4. The clear upper layer of the solution was membrane filtered (0.45µm).
5. A 10µL aliquot of filtrate was injected.

---

Figure 1-36  Analysis of Pyrogallol and Hematein in Hair Tonic

**Compounds**
1. Pyrogallol
2. Hematein

**Analytical Conditions**
- **Column**: STR ODS-II (150mm x 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium edetate buffer (pH 6.3)
  - B: Methanol
  - A / B = 9 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (280nm)

**Sample Pretreatment**
1. The sample was diluted by a factor of 100 with 10mM sodium edetate buffer (pH 6.3).
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

**Notes**
1. Guard against oxidation reactions when eluting pyrogallol and hematein, as both are easily oxidized by metal.
2. Preparation of 10mM sodium edetate buffer (pH 6.3).
Combine 3.772g of ethylenediaminetetraacetic acid disodium salt (MW = 372.24) and 200mg of sodium hydroxide (MW = 40.00), and bring up the volume to 1L with distilled water.
### Analytical Conditions

**Column**
- STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**
- A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
- B: Acetonitrile
- A / B = 3 / 1 (v/v)

**Flow Rate**: 1.0mL/min
**Temperature**: 25°C
**Detection**: Fluorescence (Ex. 230nm, Em. 345nm)

### Sample Pretreatment

1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. A 50µL aliquot of filtrate was injected.

### Notes

Absorption (210nm) is an alternate mode of detection.

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**Compounds**
1. Nalbuphine

**Analytical Conditions**

**Column**
- Shim-pack HRC-ODS (150mm × 4.6mm i.d.)

**Mobile Phase**
- A: Water
- B: Methanol
- A / B = 1 / 1 (v/v)

**Flow Rate**: 0.8mL/min
**Temperature**: 40°C
**Detection**: Absorption (254nm)
### Analytical Conditions

**Column**: STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**: A: 100 mM sodium phosphate buffer (pH 2.2) with 10mM sodium 1-octanesulfonate  
B: Methanol  
A / B = 10 / 1 (v/v)

**Flow Rate**: 1.0mL/min  
**Temperature**: 45°C  
**Detection**: Amperometry

---

**Sample Pretreatment**  
1. The sample was dissolved in mobile phase.  
2. The sample solution was membrane filtered (0.45µm).  
3. A 5µL aliquot of filtrate was injected.
Figure 1-41 Analysis of Arotinolol

- **Column**: STR ODS-M (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 20mM sodium phosphate buffer (pH 2.5) with 100mM sodium perchlorate
  B: Acetonitrile
  A / B = 3 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Fluorescence (Ex. 318nm, Em. 428nm)

**Sample Pretreatment**
1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

Figure 1-42 Analysis of Aspartic Acid and Taurine in a Medicinal Beverage

- **Column**: Shim-pack SCR-101N (300mm × 7.9mm i.d.) with guard column SCR (N) (50mm × 4.0mm i.d.)
- **Mobile Phase**: 10mM sodium citrate buffer (pH 4.5) with 1mM sodium azide
- **Flow Rate**: 1.0mL/min
- **Temperature**: 50°C
- **Detection (postcolumn derivatization method)**
  - **Reagents**: A: 20mM o-phthalaldehyde methanolic solution
    B: 200mM sodium borate buffer (pH 9.2) with 2mM β-mercaptpropionic acid
    A / B = 1 / 1 (v/v)
  - **Flow Rate**: 0.5mL/min
  - **Reaction coil**: piping part J
  - **Temperature**: 50°C
  - **Detection**: Fluorescence (Ex. 340nm, Em. 450nm)

**Sample Pretreatment**
1. The sample was diluted by a factor of 100 with mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.
### Analytical Conditions

**Column**
- STR ODS-M (150mm × 4.6mm i.d.)

**Mobile Phase**
- A: 50 mM monobasic potassium phosphate with 100mM sodium perchlorate and 50μM EDTA-2 Na
- B: Acetonitrile
- A / B = 3 / 1 (v/v)

**Flow Rate**
- 0.8mL/min

**Temperature**
- 40°C

**Detection**
- Absorption (230nm)

---

**Figure 1-43 Analysis of Tulobuterol Hydrochloride**

![Tulobuterol Analysis](image1)

**Compound**
1. Tulobuterol

---

### Analytical Conditions

**Column**
- L-Column ODS (150mm × 4.6mm i.d.)

**Mobile Phase**
- A: 1mM triethylenetetramine / 8mM phosphoric acid mixture (pH 2.6)
- B: Acetonitrile
- A / B = 1 / 1 (v/v)

**Flow Rate**
- 1.0mL/min

**Temperature**
- 40°C

**Detection**
- Absorption (240nm)

---

**Figure 1-44 Analysis of Hinokitiol**

![Hinokitiol Analysis](image2)

**Compound**
1. Hinokitiol
**Analytical Conditions**

Column : STR ODS-II (150mm × 4.6mm i.d.)

Mobile Phase : A: 10mM sodium phosphate buffer (pH 2.6)
                  B: Acetonitrile
                  A / B = 7 / 1 (v/v)

Flow Rate : 1.0mL/min

Temperature : 40°C

Detection : Fluorescence (Ex. 293nm, Em. 418nm)

---

**Compounds**

1. Methylergometrine

---

**Analytical Conditions**

Column : STR ODS-M (150mm × 4.6mm i.d.)

Mobile Phase : A: 10mM sodium phosphate buffer (pH 2.6)
                  B: Acetonitrile
                  A / B = 55 / 45 (v/v)

Flow Rate : 0.8mL/min

Temperature : 40°C

Detection : Absorption (280nm)

---

**Compounds**

1. Leukotriene D4
2. Leukotriene C4
3. Leukotriene F4
4. Leukotriene B4

---

**Figure 1-45  Analysis of Methylergometrine**

**Figure 1-46  Analysis of Leukotrienes**
2. Natural Compounds

### Analytical Conditions

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 100mM sodium 2-hydroxyisobutyrate buffer (pH 3.8)  
  B: Acetonitrile  
  A / B = 6 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (270 nm)

### Sample Pretreatment

1. The sample was membrane filtered (0.45µm).
2. A 10µL aliquot of filtrate was injected.

### Compounds

1. Sennoside A
2. Sennoside B

---

### Analytical Conditions

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6)  
  B: Acetonitrile  
  A / B = 3 / 1 (v/v)
- **Flow Rate**: 0.8mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (210 nm)

### Compound

1. 10-Hydroxy-δ-2-decenoic Acid

---

### Analytical Conditions

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6)  
  B: Acetonitrile  
  A / B = 3 / 1 (v/v)
- **Flow Rate**: 0.8mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (210 nm)
Analytical Conditions

**Column**: STR ODS-II (150mm × 4.6mm i.d.)

**Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Acetonitrile
A / B = 5 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (210nm)

Sample Pretreatment

1. The sample was crushed, added to 100mM perchloric acid, then agitated at 50°C for 30 minutes.
2. After cooling, the sample solution was centrifuged at 3000rpm for five minutes.
3. Chloroform, 1M sodium hydroxide (containing 5M sodium chloride) and 2mL of the clear upper layer of the solution were combined and well-shaken.
4. After separating, the chloroform layer was evaporated to dryness.
5. The residue was dissolved in 100mM perchloric acid.
6. A 10µL aliquot of the residue solution was injected.

Analytical Conditions

**Column**: STR ODS-M (150mm × 4.6mm i.d.)

**Mobile Phase**: A: 100mM sodium acetate buffer (pH 4.7)
B: Acetonitrile
A / B = 1 / 1 (v/v)

**Flow Rate**: 0.6mL/min

**Temperature**: 40°C

**Detection**: Fluorescence (Ex. 280nm, Em. 320nm)

Sample Pretreatment

1. The sample was diluted by a factor of 10 with methanol.
2. The sample solution was membrane filtered (0.45µm).
3. 5µL of filtrate were injected.
Figure 2-5  Analysis of Berberine in Powdered Philodendron Bark

**Analytical Conditions**

- **Column**: Shim-pack VP-ODS (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 200mM sodium perchlorate
  - B: Acetonitrile
  - A / B = 5 / 3 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (345nm)

**Sample Pretreatment**

1. The sample was diluted with 100mM hydrochloric acid / acetonitrile [1 / 1 (v/v)] solution.
2. The sample solution was membrane filtered (0.45µm).
3. A 5µL aliquot of filtrate was injected.

1. Berberine

Figure 2-6  Analysis of Glycyrrhizic Acid in Powdered Glycyrrhiza

**Analytical Conditions**

- **Column**: STR ODS-II (150mm × 4.0mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6)
  - B: Acetonitrile
  - A / B = 3 / 2 (v/v)
- **Flow Rate**: 0.6mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (254nm)

**Sample Pretreatment**

1. The sample was dissolved in dilute ethanol and extracted.
2. The extracted solution was membrane filtered (0.45µm).
3. A 5µL aliquot of filtrate was injected.

**Compound**

1. Glycyrrhizic acid

Figure 2-7  Analysis of Glycyrrhizic Acid in Powdered Glycyrrhiza
3. Antibiotics

Figure 3-1  Analysis of Amantadine

- **Analytical Conditions**
  - **Column**: STR ODS-II (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: 20mM sodium citrate buffer (pH 4.5) with 100mM sodium perchlorate
    - B: Methanol
    - A / B = 3 / 2 (v/v)
  - **Flow Rate**: 0.6mL/min
  - **Temperature**: 40°C
  - **Detection (postcolumn derivatization method)**
    - **Reagents**:  
      - A: 200mM sodium borate buffer (pH 9.2) with 1mM β-mercaptoacetic acid
      - B: 20mM o-phthalaldehyde methanolic solution
      - A / B = 4 / 1 (v/v)
    - **Flow Rate**: 0.3mL/min
    - **Reaction coil**: SUS tubing (2.0 m × 0.5mm i.d.)
    - **Temperature**: 80°C
    - **Detection**: Fluorescence (Ex. 330nm, Em. 460nm)
  - **Sample Pretreatment**
    1. The sample was dissolved in 100mM hydrochloric acid.
    2. 10µL of sample solution were injected.

![Amantadine Chromatogram](image1.png)

Figure 3-2  Analysis of Actinomycin D

- **Analytical Conditions**
  - **Column**: STR ODS-M (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
    - B: Methanol
    - A / B = 1 / 4 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 50°C
  - **Detection**: Absorption (240nm)
  - **Sample Pretreatment**
    1. The sample was diluted with methanol.
    2. The diluted solution was membrane filtered (0.45µm).
    3. 5µL of filtrate were injected.

![Actinomycin D Chromatogram](image2.png)
### Analytical Conditions

**Column**: STR ODS-M (150mm × 6.0mm i.d.)

**Mobile Phase**: A: 1M imidazole acetate buffer (pH 7.1) with 50mM magnesium acetate and 1mM EDTA-2 Na  
B: Methanol  
A / B = 4 / 1 (v/v)

**Flow Rate**: 0.6mL/min  
**Temperature**: 40°C  
**Detection**: Fluorescence (Ex. 380nm, Em. 520nm)

### Notes

Preparation of 1M Imidazole Acetate Buffer (pH 7.1)  
Mix 68.08g of imidazole (MW = 68.08, d = 1.0303) and 28.7mL of glacial acetic acid (MW = 60.05, d = 1.0492), and bring up the volume to 1L with distilled water.

---

### Analytical Conditions

**Column**: STR ODS-M (150mm × 4.6mm i.d.)

**Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate  
B: Methanol  
A / B = 1 / 1 (v/v)

**Flow Rate**: 1.0mL/min  
**Temperature**: 50°C  
**Detection**: Absorption (230nm)

### Sample Pretreatment

1. The sample was dissolved in methanol.  
2. A 10µL aliquot of solution was injected.
Figure 3-5  Analysis of Mitomycin C

Analytical Conditions

Column: STR ODS-M (150mm × 4.6mm i.d.)
Mobile Phase: A: 10mM Sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Methanol
A / B = 3 / 1 (v/v)
Flow Rate: 1.0mL/min
Temperature: 50°C
Detection: Absorption (360nm)

Sample Pretreatment

1. The sample was dissolved in enough methanol to make a 20mg/mL sample solution.
2. 5μL of solution were injected.

Figure 3-6  Analysis of Rifampicin

Analytical Conditions

Column: STR ODS-M (150mm × 4.6mm i.d.)
Mobile Phase: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate
B: Methanol
A / B = 1 / 3 (v/v)
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: Absorption (230nm)
4. Other Pharmaceutical Chemicals

- **Analytical Conditions**
  - **Column**: STR ODS-II (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 7.0)
    - B: Acetonitrile
    - A / B = 8 / 2 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 40°C
  - **Detection**: Absorption (254nm)

- **Sample Pretreatment**
  1. The sample was dissolved in mobile phase.
  2. The sample solution was membrane filtered (0.45µm).
  3. A 10µL aliquot of filtrate was injected.

---

- **Compounds**
  1. Saccharin
  2. Glycyrrhizic Acid

---

- **Analytical Conditions**
  - **Column**: STR ODS-II (150mm × 4.6mm i.d.)
  - **Mobile Phase**: A: Water
    - B: Acetonitrile
    - A / B = 65 / 35 (v/v)
  - **Flow Rate**: 1.0mL/min
  - **Temperature**: 40°C
  - **Detection**: Absorption (312nm)

- **Sample Pretreatment**
  1. The sample was dissolved in mobile phase.
  2. The sample solution was membrane filtered (0.45µm).
  3. A 10µL aliquot of filtrate was injected.

---

- **Compounds**
  1. Flubendazole

---

**Figure 4-1** Analysis of Saccharin and Glycyrrhizic Acid

**Figure 4-2** Analysis of Flubendazole
Figure 4-3  Analysis of Zeranol

**Analytical Conditions**

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: Water  
  B: Acetonitrile  
  A / B = 6 / 5 (v/v)
- **Flow Rate**: 0.8mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (263nm)

**Sample Pretreatment**

1. The sample was dissolved in mobile phase.
2. The sample solution was membrane filtered (0.45µm).
3. A 10µL aliquot of filtrate was injected.

---

Figure 4-4  Analysis of Retinol, Tocopherol and ß-Carotene

**Analytical Conditions**

- **Column**: STR ODS-II (250mm × 4.6mm i.d.)
- **Mobile Phase**: A: Acetonitrile  
  B: Ethanol  
  A / B = 1 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 45°C
- **Detection**: Absorption (210-550nm; photo diode array)
### Analytical Conditions

**Figure 4-5** Analysis of a Nicotinic Acid, Nicotinamide, Riboflavin Phosphate, Pyridoxine, Caffeine, Thiamine and Riboflavin

**Column**: STR ODS-II (250mm × 4.6mm i.d.)

**Mobile Phase**:  
A: 10mM Sodium phosphate buffer (pH 2.6) with 5mM sodium 1-heptanesulfonate  
B: Acetonitrile  
A / B = 9 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (270nm)

### Compounds
1. Nicotinic Acid  
2. Nicotinamide  
3. Riboflavin Phosphate  
4. Pyridoxine  
5. Caffeine  
6. Thiamine  
7. Riboflavin

---

**Figure 4-6** Analysis of Nicotinamide, Riboflavin Phosphate, Pyridoxine, Caffeine, and Thiamine in a Medicinal Beverage

**Analytical Conditions**

**Column**: Shim-pack VP-ODS (150mm × 4.6mm i.d.)

**Mobile Phase**:  
A: 20mM sodium phosphate buffer (pH 2.5) with 10mM sodium 1-hexanesulfonate  
B: Acetonitrile  
A / B = 9 / 1 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (270nm)

### Sample Pretreatment

1. The sample was diluted with mobile phase.  
2. The sample solution was membrane filtered (0.45µm).  
3. 5µL of filtrate were injected.

### Notes

The different compounds contained in this sample have different maximum absorption wavelengths. The range of wavelengths spans from 250-290nm; 270nm, the median wavelength, was used to obtain Figure 4-6.
### Analytical Conditions

#### Column
 STR ODS-II (250mm × 4.6mm i.d.)

#### Mobile Phase
- **A:** 100mM sodium tartrate buffer (pH 4.3) with 10mM sodium 1-hexanesulfonate
- **B:** Methanol
  - A / B = 2 / 1 (v/v)

#### Flow Rate
 1.0mL/min

#### Temperature
50°C

#### Detection (postcolumn derivatization method)

- **Reagents:**
  - A: 400mM sodium borate buffer (pH 9.2)
  - B: 10mM o-phthalaldehyde methanol solution
  - A / B = 4 / 1 (v/v)

- **Flow Rate:** 0.5mL/min
- **Reaction coil:** piping part J
- **Temperature:** 50°C
- **Detection:** Fluorescence (Ex. 360nm, Em. 440nm)

### Sample Pretreatment

1. 100mg of sample and 100mL of mobile phase were combined and vortexed.
2. The sample solution was heated at 50°C for one hour.
3. The solution was membrane filtered (0.45µm).
4. 10µL of filtrate were injected.
Figure 4-9  Analysis of Urea

**Analytical Conditions**

- **Column**: Asahipak NH2P-50 4E (250mm × 4.6mm i.d.)
- **Mobile Phase**: A: 20mM sodium phosphate buffer (pH 2.5)  
  B: Acetonitrile  
  A / B = 1 / 3 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (200nm)

**Notes**

Before analysis, condition the column with 100mM sodium phosphate buffer (pH 2.2) at a flow rate of 1.0mL/min for approximately 30 minutes to acidify the amino residue on the packing. The flushing procedure is not required again unless the column is used under different analytical conditions.

Figure 4-10  Analysis of Acetic and Carbonic Acids in a Dialytic Solution

**Compounds**

1. Acetic Acid  
2. Carbonic Acid

**Analytical Conditions**

- **Column**: Shim-pack SCR-102H (300mm × 8.0mm i.d.) with guard column SCR-102H (50mm × 6.0mm i.d.)
- **Mobile Phase**: 2mM p-toluenesulfonic acid
- **Flow Rate**: 0.8mL/min
- **Temperature**: 40°C
- **Detection**: Electroconductivity (post column pH buffering method)

**Sample Pretreatment**

1. The sample was diluted by a factor of 10 with distilled water.  
2. The diluted sample was membrane filtered (0.45µm).  
3. 10µL of filtrate were injected.
Figure 4-11 Analysis of Urea, Creatinine and Cyanocobalam (Vitamin B12)

**Analytical Conditions**

**Column**: Asahipak GS-310H (250mm × 7.6mm i.d.)

**Mobile Phase**: A: 20mM sodium phosphate buffer (pH 2.5)
B: Acetonitrile
A / B = 1 / 3 (v/v)

**Flow Rate**: 1.0mL/min

**Temperature**: 40°C

**Detection**: Absorption (200nm)

**Compounds**

1. Urea
2. Creatinine
3. Vitamin B12

---

Figure 4-12 Analysis of Glucuronic, Mannuronic and Galacturonic Acids

**Analytical Conditions**

**Column**: Shim-pack SCR-101N (300mm × 7.8mm i.d.) with guard column SCR (N) (50mm × 4.0mm i.d.)

**Mobile Phase**: 20mM sodium phosphate buffer (pH 2.5)

**Flow Rate**: 1.0mL/min

**Temperature**: 50°C

**Detection**: Absorption (210nm)

**Notes**

Before analysis, condition the column with 100mM sodium phosphate buffer (pH 2.2) at a flow rate of 0.6mL/min, for approximately one hour. The flushing procedure is not required again unless the column is used under different analytical conditions.
Figure 4-13 Analysis of Glycyrrhizic Acid and Hinokitiol

**Analytical Conditions**

- **Column**: STR ODS-II (150mm x 4.6mm i.d.)
- **Mobile Phase**: A: 1mM triethylenetetramine 8mM phosphoric acid  
  B: Acetonitrile  
  A / B = 3 / 2 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (245nm)

**Compounds**

1. Glycyrrhizic acid  
2. Hinokitiol

Figure 4-14 Analysis One of Carnitine Chloride in a Medicinal Beverage

**Analytical Conditions**

- **Column**: STR ODS-II (150mm x 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 5mM sodium 1-decanesulfonate  
  B: Acetonitrile  
  A / B = 5 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (210nm)

**Sample Pretreatment**

1. The sample was membrane filtered (0.45µm).  
2. 10µL of filtrate were injected.
### Analytical Conditions

**Compound**

1. Carnitine

- **Column**: Shim-pack IC-C3 (100mm × 4.6mm i.d.)
- **Mobile Phase**: 5.3mM oxalic acid
- **Flow Rate**: 1.2mL/min
- **Temperature**: 40°C
- **Detection**: Electroconductivity

**Sample Pretreatment**

1. The sample was diluted by a factor of 10 with distilled water.
2. The diluted solution was membrane filtered (0.45µm).
3. 10µL of filtrate were injected.

---

### Analytical Conditions

**Compound**

1. Didecyl(dimethyl)ammonium

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 20mM sodium phosphate buffer (pH 2.5) with 500mM sodium 2-naphthalenesulfonate  
  B: Methanol  
  A / B = 1 / 4 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (275nm)
**Figure 4-17  Analysis of Furosemide in Urine**

**Analytical Conditions**

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 20mM sodium phosphate buffer (pH 2.5)  
  B: Acetonitrile  
  A / B = 2 / 1 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (340nm)

**Sample Pretreatment**

1. The sample was membrane filtered (0.45µm).
4. A 10µL aliquot of filtrate was injected.

**Compounds**

1. Furosemide

![Figure 4-17 Analysis of Furosemide in Urine](image)

---

**Figure 4-18  Analysis of Phospholipids**

**Analytical Conditions**

- **Column**: Shim-pack CLC-SIL(M) (250mm × 4.6mm i.d.)
- **Mobile Phase**: A: 10mM sodium phosphate buffer (pH 2.6) with 100mM sodium perchlorate  
  B: Acetonitrile  
  A / B = 1 / 6 (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (205nm)

**Notes**

When the sample is dissolved in chloroform without further dilution, a ghost peak appears at two minutes, preventing the quantification of phosphatidal-ethanolamine and phosphatidal-inositol. Avoid this problem by dissolving the sample in a small amount of chloroform, then diluting it with ethanol.

**Compounds**

1. Phosphatidal-Ethanolamine
2. Phosphatidal-Inositol
3. Phosphatidal-Serine
4. Phosphatidal-Choline
5. Sphingomyelins

![Figure 4-18 Analysis of Phospholipids](image)
Figure 4-19 Analysis of Ascorbyl Phosphate (magnesium salt)

Analytical Conditions
- Column: STR ODS-M (150mm × 4.6mm i.d.)
- Mobile Phase: 10mM phosphoric acid with 5mM triethylamine
- Flow Rate: 1.0mL/min
- Temperature: 40°C
- Detection: Absorption (240nm)

Sample Pretreatment
1. The sample was dissolved in enough distilled water to make a 100nmol/mL sample solution.
2. A 10µL aliquot of solution was injected.

Figure 4-20 Analysis of Cholecalciferol (Vitamin D3) and α-Tocopherol (Vitamin E) Acetate

Analytical Conditions
- Column: STR ODS-M (150mm × 4.6mm i.d.)
- Mobile Phase: Water / Methanol = 1 / 20 (v/v)
- Flow Rate: 1.0mL/min
- Temperature: 40°C
- Detection: Absorption (240nm)

Sample Pretreatment
1. The sample was dissolved in ethanol.
2. 10µL of sample solution were injected.
Figure 4-21  Analysis of Methylmethioninesulfonium (Vitamin U)

**Analytical Conditions**

- **Column**: Asahipak ES-502C (100mm × 6.0mm i.d.)
- **Mobile Phase**: A: 50mM sodium citrate buffer (pH 5.7)
  B: Methanol
  \( A / B = 4 / 1 \) (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 50°C

**Detection (postcolumn derivatization method)**

- **Reagents**: A: 500mM sodium borate buffer (pH 9.2) with 2mM \( \beta \)-mercaptopropionic acid
  B: 20mM \( o \)-phthalaldehyde methanolic solution
  \( A / B = 4 / 1 \) (v/v)
- **Flow Rate**: 0.5mL/min
- **Reaction coil**: piping part J
- **Temperature**: 50°C
- **Detection**: Fluorescence (Ex. 340nm, Em.450nm)

Figure 4-22  Analysis of Bilirubin

**Analytical Conditions**

- **Column**: STR ODS-II (150mm × 4.6mm i.d.)
- **Mobile Phase**: A: 100mM sodium acetate buffer (pH 4.7)
  B: Methanol
  \( A / B = 1 / 20 \) (v/v)
- **Flow Rate**: 1.0mL/min
- **Temperature**: 40°C
- **Detection**: Absorption (450nm)
Analytical Conditions

Column: Asahipak NH2P-50 (250mm × 4.6mm i.d.)
Mobile Phase: A: 10mM sodium phosphate buffer (pH 7.0)
              B: Acetonitrile
              A / B = 3 / 7 (v/v)
Flow Rate: 0.8mL/min
Temperature: 40°C

Detection (postcolumn derivatization method)
Reagents: Reagent kit for Shimadzu Amino Acid Analysis System
Flow Rate: 0.3mL/min
Temperature: 40°C
Detection: Fluorescence (Ex. 348nm, Em. 450nm)

Sample Pretreatment
1. 1mL of sample and 1mL of 1N hydrogen chloride were combined.
2. The sample solution was heated at 110°C for one hour, and the pantothenic acid in the sample was hydrolyzed to β-alanine.
3. The hydrolyzed solution was membrane filtered (0.45µm).
4. A 5µL aliquot of filtrate was injected.

Analytical Conditions

Column: STR ODS-II (150mm × 4.6mm i.d.)
Mobile Phase: A: 10mM phosphoric acid with 15mM tributylamine
              B: Acetonitrile
              A / B = 5 / 1 (v/v)
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: Absorption (258nm)

Compound

1. β-Alanine

Figure 4-23 Analysis of β-Alanine (from Calcium Pantothenate) in a Medicinal Beverage

Compound

1. Iron edetate

Figure 4-24 Analysis of Iron Edetate
(Ethlenediaminetetraacetic Acid, Fe)
Analytical Conditions

Column: STR ODS-II (150mm x 4.6mm i.d.)
Mobile Phase: Gradient elution
  A: 10mM sodium phosphate buffer (pH 7.0)
  B: Acetonitrile
Flow Rate: 1.0mL/min
Temperature: 40°C
Detection: Absorption (254nm)

Gradient Program

<table>
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<th>Time (minutes)</th>
<th>Conc. of B (%)</th>
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<td>5</td>
</tr>
<tr>
<td>20.00</td>
<td>35</td>
</tr>
<tr>
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<td>80</td>
</tr>
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Sample Pretreatment

1. 100µL of 100mM phenylisothiociane (acetonitrile solution) and 100µL of 1M triethylamine (acetonitrile solution) were added to 200µL of sample.
2. The sample solution was allowed to stand at room temperature.
3. 400mL of hexane were added to the solution and vortexed.
4. After separating, the hexane layer was membrane filtered (0.45µm).
5. 4µL of filtrate were injected.
5-1 : Sample Pretreatment

To obtain stable, sensitive chromatograms, an HPLC sample must often be pretreated before analysis. Pretreatment procedures vary from extraction of target components to filtration to avoid column clogging. Section 5.1 discusses the typical HPLC sample pretreatment methods.

(1) Dissolving and Extracting

When analyzing a solid sample with HPLC, it must first be dissolved, preferably in a solvent similar to the mobile phase. Occasionally, a liquid extraction with a separatory funnel or solid extraction with a mini-column is performed. For some procedures, the sample is dissolved in a small amount of solvent, then diluted with mobile phase. Carefully examine chromatograms in which the solvent differs greatly from the mobile phase because ghost peaks or peak distortion may be present.

(2) Concentrating and Diluting

The concentration of the sample may need adjustment to accommodate detector sensitivity or column efficiency. Preferably, the mobile phase or a similar solvent is used for dilutions.

(3) Elimination of Minute Particles

Sample solutions containing minute particles usually clog the column or tubing. Eliminate particulate matter with a centrifuge or by filtration.

   a. Centrifuge

   A centrifuge at 3,000-12,000 rpm for three to five minutes removes minute particles from a solution. This technique is especially useful when the particles clog a membrane filter.

   b. Membrane Filtration

   Membrane filtration is the primary method used for particle elimination. Disposable membrane filters with pore sizes from 0.2-0.5 μm (0.45 μm is common for HPLC) eliminate most particles from a solution, preventing clogs in the column or tubing. Different filter types are used for water and organic solvents; some are specifically available for ion chromatography, some for HPLC. The procedures in Pharmaceutical Application Data use the filter with a plastic syringe. The pointed tip of the filter accommodates a needle for injections. Proper knowledge of membrane filtration is required to assure removal of particulate matter.

(4) Protein Elimination

When not the target component, proteins must be eliminated from a sample to prevent protein adsorption clogs in the column. Organic solvents, acids and bases are commonly used for protein removal. Commercial ultrafiltration membranes, sold as disposable cartridges, succeed at eliminating 5,000-10,000MW proteins.
5-2 : Preparation of Buffers

100mM Sodium Phosphate Buffer (pH 2.2)
monobasic sodium phosphate • 2H₂O (MW = 156.01) 50mmol (7.8g)
phosphoric acid (85%; 14.7mol/L) 50mmol (3.4mL)
Combine and bring the total volume to 1L with distilled water.

50mM Sodium Phosphate Buffer (pH 2.8)
monobasic sodium phosphate • 2H₂O (MW = 156.01) 40mmol (6.24g)
phosphoric acid (85%; 14.7mol/L) 10mmol (0.68mL)
Combine and bring the total volume to 1L with distilled water.

10mM Sodium Phosphate Buffer (pH 2.6)
monobasic sodium phosphate • 2H₂O (MW = 156.01) 5mmol (0.78g)
phosphoric acid (85%; 14.7mol/L) 5mmol (0.34mL)
Combine and bring the total volume to 1L with distilled water, or
dilute 100mM sodium phosphate buffer (pH 2.2) by a factor of ten with distilled water.

100mM Sodium Phosphate Buffer (pH 6.8)
monobasic sodium phosphate • 2H₂O (MW = 156.01) 50mmol (7.8g)
dibasic sodium phosphate • 12H₂O (MW = 358.14) 50mmol (17.9g)
Combine and bring the total volume to 1L with distilled water.

10mM Sodium Phosphate Buffer (pH 7.0)
monobasic sodium phosphate • 2H₂O (MW = 156.01) 5mmol (0.78g)
dibasic sodium phosphate • 12H₂O (MW = 358.14) 5mmol (1.79g)
Combine and bring the total volume to 1L with distilled water, or
dilute 100mM sodium phosphate buffer (pH 6.8) by a factor of ten with distilled water.

20mM Sodium Citrate Buffer (pH 3.1)
citric acid • H₂O (MW = 210.14) 16.7mmol (3.51g)
sodium citrate • 2H₂O (MW = 294.10) 3.3mmol (0.97g)
Combine and bring the total volume to 1L with distilled water.

20mM Sodium Citrate buffer (pH 4.5)
citric acid • H₂O (MW = 210.14) 10mmol (2.1g)
sodium citrate • 2H₂O (MW = 294.10) 10mmol (2.94g)
Combine and bring the total volume to 1L with distilled water.

10mM Sodium Tartrate Buffer (pH 3.0)
tartaric acid (MW = 150.09) 7.5mmol (1.13 g)
sodium tartrate • 2H₂O (MW = 230.08) 2.5mmol (0.58 g)
Combine and bring the total volume to 1L with distilled water.

10mM Sodium Tartate Buffer (pH 4.3)
tartaric acid (MW = 150.09) 2.5mmol (0.375g)
sodium tartrate • 2H₂O (MW = 230.08) 7.5mmol (1.726g)
Combine and bring the total volume to 1L with distilled water.
**20mM Ethanolamine Acetate buffer (pH 9.6)**

ethanolamine (MW = 61.08; d = 1.017) 20mmol (1.22mL)
glacial acetic acid (99.5%; 17.4mol/L) 10mmol (0.575mL)
Combine and bring the total volume to 1L with distilled water.

**10mM Sodium Acetate Buffer (pH 4.7)**

glacial acetic acid (99.5%; 17.4mol/L) 5mmol (0.287mL)
sodium acetate • 3H₂O (MW = 136.08) 5mmol (0.680g)
Combine and bring the total volume to 1L with distilled water.

**100mM Potassium Borate Buffer (pH 9.2)**

boric acid (MW = 61.83) 100mmol (6.18g)
potassium hydroxide (MW = 56.11) 50mmol (2.81g)
Combine and bring the total volume to 1L with distilled water.

**100mM Sodium Borate Buffer (pH 9.2)**

boric acid (MW = 61.83) 100mmol (6.18g)
sodium hydroxide (MW = 40.00) 50mmol (2.00g)
Combine and bring the total volume to 1L with distilled water.
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