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Fast Method Scouting for Chiral Separation Utilizing CHIRAL ART Columns

CHIRAL ART are HPLC columns with polysaccharide derivatives chiral selector, and are suitable for separations of wide range of chiral compounds. On CHIRAL ART Immobilized type with high solvent versatility, chromatographers can freely choose the most suitable mobile phase by considering the solubility and resolution of the target compounds. The excellent separation of various racemic compounds was achieved through fast method scouting utilizing the short columns and 3 µm CHIRAL ART immobilized type with three different chiral selectors.

Method scouting

- Scouting protocol

**Column**

CHIRAL ART 3 µm, 50 X 3.0 mmL.D.
Amylose-SA
Cellulose-SB
Cellulose-SC

**Mobile phase**

<table>
<thead>
<tr>
<th>Eluent A</th>
<th>Eluent B</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-propanol</td>
<td>2-propanol</td>
</tr>
<tr>
<td>ethanol</td>
<td>ethanol</td>
</tr>
<tr>
<td>ethyl acetate</td>
<td>ethanol</td>
</tr>
</tbody>
</table>

Gradient 5-50% Eluent B

**Additive**

Acidic compounds
- TFA, Acetic acid, Formic acid, etc.

Basic compounds
- DEA, Butylamine, Ethanolamine, etc.

Zwitterionic compounds
- Both or either of acidic additive and basic additive

Nonionic compounds
- None

*Usually 0.1% (upper limit 0.5%)

**Experimental Matrix**

<table>
<thead>
<tr>
<th>mode</th>
<th>NP</th>
<th>PO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eluent A</td>
<td>n-hexane MTBE</td>
<td>ethyl acetate</td>
</tr>
<tr>
<td>Eluent B</td>
<td>2-propanol</td>
<td>ethanol</td>
</tr>
<tr>
<td>additive</td>
<td>0.1% DEA</td>
<td></td>
</tr>
</tbody>
</table>

Amphole-SA
Cellulose-SB
Cellulose-SC

Select

**Hydroxychloroquine**

Column : 3 µm, 50 X 3.0 mmL.D.
Flow rate : 0.85 mL/min
Gradient : 5%B (0-0.5 min), 5-50%B (0.5-1.5 min), 50%B (1.5-2.0 min) for normal phase mode
- 0%B (0-0.5 min), 0-20%B (0.5-1.5 min), 20%B (1.5-2.0 min) for polar organic mode
Temperature : 25°C
Detection : UV at 265, 290, 334 nm
Injection : 2 µL (150 mg/mL)

The baseline resolution is achieved under four conditions in the method scouting for hydroxychloroquine. The combination of Amylose-SA phase and MTBE/ethanol containing 0.1% DEA is selected as the most favorable condition in consideration of retention and resolution.
Separation results under simple isocratic conditions optimized through scouting of each compound

The combination of the short columns packed with three types of 3 µm particles and the rapid gradient elution of eight types of Normal Phase (NP) and Polar Organic (PO) mobile phase are employed for separation method scouting of pharmaceutical compounds below. The selected conditions from scouting with gradient elution for each compound are converted to the isocratic elution methods and optimized.

- The fast method development chiral separations is allowed through the method scouting.
- The selected conditions from scouting with gradient elution for each compound are converted to the isocratic elution methods and optimized to achieve the ultra-fast separation method within 2 minutes.
Chiral method optimization of ionic compounds utilizing immobilized type column in reversed phase mode ~ Influence of pH on retention behavior ~

Features of immobilized type columns

Immobilized type CHIRAL ART columns can be used with various solvents that are commonly used for HPLC analysis. They can be used in both normal phase mode and reversed phase mode as they are compatible with non-aqueous and aqueous solvents (*).

Reversed phase mode will be effective in case where a sample is hydrophilic and has limited solubility in organic solvent (e.g. Hexane). In this technical data sheet, we will introduce influence of pH of mobile phase on retention behavior of ionic compounds. We also introduce several example of chiral separation on reversed phase mode.

Specifications (Immobilized type)

<table>
<thead>
<tr>
<th>Column/Packing material</th>
<th>Particle size (µm)</th>
<th>Chiral selector</th>
<th>Usable pH range</th>
<th>Temperature range</th>
<th>Pressure limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHIRAL ART Amylose-SA</td>
<td>3</td>
<td>Amylose tris(3,5-dimethylphenylcarbamate)</td>
<td>2.0-9.0</td>
<td>0-40°C</td>
<td>30 MPa</td>
</tr>
<tr>
<td>CHIRAL ART Cellulose-SB</td>
<td>5, 10</td>
<td>Cellulose tris(3,5-dimethylphenylcarbamate)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHIRAL ART Cellulose-SC</td>
<td>20</td>
<td>Cellulose tris(3,5-dichlorophenylcarbamate)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Usable mobile phase

- Normal phase: n-hexane, n-heptane, methanol, ethanol, 2-propanol, acetonitrile, ethyl acetate, tetrahydrofuran, chloroform, t-butyl methyl ether, etc.
- Reversed phase: acetonitrile, methanol, ethanol, 2-propanol, tetrahydrofuran, water, aqueous buffer, etc.

Tips for optimizing chiral separation method of ionic compounds on reversed phase mode

- **Mobile phase** > Optimal pH that ionization of analyte is suppressed is recommended. (Retention will be extended and possibility of greater resolution is expected.)
- **Column** > Immobilized type CHIRAL ART columns are the best option.

Reversed phase analyses with optimized separation methods

**Warfarin (pKa 5.56)**

- Column: CHIRAL ART Cellulose-SB
- Eluent: 20 mM phosphoric acid (pH 2.1)/acetonitrile (50/50)

**Donepezil (pKa 8.62)**

- Column: YMC CHIRAL Cellulose-SB
- Eluent: 20 mM NH₄HCO₃-DEA (pH 9.0)/acetonitrile (40/60)
Retention behavior of acid compound on reversed phase mode

- **Influence of pH on retention behavior**

  - **Warfarin** (pKa 5.56)
    - Column: CHIRAL ART Cellulose-SB 5 µm
    - Eluent: 20 mM phosphate buffer/acetonitrile (50/50)
    - Flow rate: 1.0 mL/min
    - Temperature: 25°C
    - Detection: UV at 254 nm
    - Injection: 2 µL (0.2 mg/mL)

  ![Retention of Warfarin](image)

  - For acidic compound, retention is extended and good separation is achieved when decreasing the pH of a mobile phase (ionization was suppressed).

Retention behavior of basic compound on reversed phase mode

- **Influence of pH on retention behavior**

  - **Donepezil** (pKa 8.62)
    - Column: CHIRAL ART Cellulose-SB 5 µm
    - Eluent: 20 mM phosphate buffer/acetonitrile (40/60)
    - Flow rate: 1.0 mL/min
    - Temperature: 25°C
    - Detection: UV at 254 nm
    - Injection: 2 µL (0.2 mg/mL)

  ![Retention of Donepezil](image)

  - For basic compound, retention is extended and good separation is achieved when increasing the pH of a mobile phase (ionization was suppressed).
**HPLC Analytical Data**

### N-CBZ-DL-アラニン
N-CBZ-DL-Alanine

- **Column**: CHIRAL ART Amylose-C Neo (5 µm)
- **Eluent**: n-hexane/2-propanol/TFA (80/20/0.1)
- **Flow rate**: 1.0 mL/min
- **Temperature**: 25°C
- **Detection**: UV at 254 nm
- **Injection**: 10 µL (1 mg/mL)

**α** 2.2  
**Rs** 12.4

### N-CBZ-DL-アラニン
N-CBZ-DL-Alanine

- **Column**: CHIRAL ART Cellulose-C (5 µm)
- **Eluent**: n-hexane/2-propanol/TFA (80/20/0.1)
- **Flow rate**: 1.0 mL/min
- **Temperature**: 25°C
- **Detection**: UV at 254 nm
- **Injection**: 10 µL (1 mg/mL)

**α** 3.0  
**Rs** 16.4

### DL-アミノグルテチミド
DL-Aminoglutethimide

- **Column**: CHIRAL ART Cellulose-SJ (5 µm)
- **Eluent**: n-hexane/ethyl acetate/diethylamine (70/30/0.1)
- **Flow rate**: 1.0 mL/min
- **Temperature**: 25°C
- **Detection**: UV at 254 nm
- **Injection**: 5 µL (1 mg/mL)

**α** 1.25  
**Rs** 4.18

### アトロピン
Atropine

- **Column**: CHIRAL ART Amylose-C (5 µm)
- **Eluent**: n-hexane/ethanol/ethanolamine (87/13/0.1)
- **Flow rate**: 1.0 mL/min
- **Temperature**: 25°C
- **Detection**: UV at 230 nm
- **Injection**: 2 µL (1 mg/mL)
**Atropine**

Column: CHIRAL ART Cellulose-C (5 µm)
250 X 4.6 mm I.D.
Eluent: n-hexane/ethanol/ethanolamine (90/10/0.1)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 230 nm
Injection: 2 µL (1 mg/mL)

**Benzoin**

Column: CHIRAL ART Amylose-C (5 µm)
250 X 4.6 mm I.D.
Eluent: n-hexane/2-propanol (90/10)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 254 nm
Injection: 10 µL (0.1 mg/mL)

**1,1'-Binaphthyl-2,2'-diol**

Column: CHIRAL ART Amylose-SA (5 µm)
250 X 4.6 mm I.D.
Eluent: n-hexane/2-propanol (50/50)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 240 nm
Injection: 10 µL (0.025 mg/mL)
Sample: Supplied by Tokyo Chemical Industry Co., Ltd.
1,1'-Binaphthyl-2,2'-diyl Bis(trifluoromethanesulfonate) E170901B

Column : CHIRAL ART Cellulose-SB (5 µm)  
250 X 4.6 mmI.D.  
Eluent : n-hexane/methyl tert-butyl ether (100/2)  
Flow rate : 1.0 mL/min  
Temperature : 25 °C  
Detection : UV at 285 nm  
Injection : 10 µL (0.5 mg/mL)  
Sample : Supplied by Tokyo Chemical Industry Co., Ltd.

2,6-Bis(4-isopropyl-2-oxazolin-2-yl)pyridine E170727B

Column : CHIRAL ART Cellulose-C (5 µm)  
250 X 4.6 mmI.D.  
Eluent : n-hexane/2-propanol (60/40)  
Flow rate : 1.0 mL/min  
Temperature : 25 °C  
Detection : UV at 285 nm  
Injection : 10 µL (0.5 mg/mL)  
Sample : Supplied by Tokyo Chemical Industry Co., Ltd.

1,2-Bis[(2-methoxyphenyl)phenylphosphino]ethane T180515B

Column : CHIRAL ART Amylose-C Neo (5 µm)  
250 X 4.6 mmI.D.  
Eluent : n-hexane/2-propanol (90/10)  
Flow rate : 1.0 mL/min  
Temperature : 25 °C  
Detection : UV at 285 nm  
Injection : 10 µL (0.5 mg/mL)  
Sample : Supplied by Tokyo Chemical Industry Co., Ltd.

1,2-Bis[(2-methoxyphenyl)phenylphosphino]ethane H170821E

Column : CHIRAL ART Cellulose-SJ (5 µm)  
250 X 4.6 mmI.D.  
Eluent : n-hexane/chloroform (80/20)  
Flow rate : 1.0 mL/min  
Temperature : 25 °C  
Detection : UV at 290 nm  
Injection : 5 µL (1.0 mg/mL)
2,6-ビス(4-フェニル-2-オキサゾリン-2-イル)ピリジン
2,6-Bis(4-phenyl-2-oxazolin-2-yl)pyridine

Column : CHIRAL ART Cellulose-C (5 µm)
250 X 4.6 mm I.D.
Eluent : n-hexane/ethanol (40/60)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 285 nm
Injection : 10 µL (0.5 mg/mL)
Sample : Supplied by Tokyo Chemical Industry Co., Ltd.

セチリジン（レボセチリジン）
Cetirizine (Levocetirizine)

Column : CHIRAL ART Cellulose-SB (3 µm)
150 X 4.6 mm I.D.
Eluent : acetonitrile/formic acid/diethylamine (100/0.1/0.1)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 230 nm
Injection : 2 µL (200 µg/mL)

クロロキン
Chloroquine

Column : CHIRAL ART Amylose-SA (5 µm)
250 X 4.6 mm I.D.
Eluent : MTBE*/ethanol/diethylamine (95/5/0.1)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 344 nm
Injection : 5 µL (100 µg/mL)

* methyl tert-butyl ether
**3,3'-ジブロモ-1,1'-ビ-2-ナフトール**
3,3'-Dibromo-1,1'-bi-2-naphthol

Column: CHIRAL ART Amylose-SA (5 μm)
Eluent: n-hexane/ethanol (75/25)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 285 nm
Injection: 10 μL (0.5 mg/mL)

**2,2'-ジメトキシ-1,1'-ビナフチル**
2,2'-Dimethoxy-1,1'-binaphthyl

Column: CHIRAL ART Amylose-SA (5 μm)
Eluent: n-hexane/2-propanol (70/30)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 290 nm
Injection: 10 μL (0.5 mg/mL)

**ドネペジル**
Donepezil

Column: CHIRAL ART Cellulose-C (5 μm)
Eluent: n-hexane/ethanol/ethylenediamine (85/15/0.1)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 271 nm
Injection: 10 μL (20 μg/mL)
ドネペジル
Donepezil

Donepezil F140707A

Column : CHIRAL ART Cellulose-SB (5 µm)
Eluent : 20 mM NH₄HCO₃-diethylamine (pH 9.0)/acetonitrile (40/60)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 254 nm
Injection : 2 µL (200 µg/mL)

デュロキセチン塩酸塩
Duloxetine hydrochloride

Duloxetine hydrochloride F131003A

Column : CHIRAL ART Amylose-C (5 µm)
Eluent : n-hexane/ethanol/diethylamine (95/5/0.2)
Flow rate : 1.0 mL/min
Temperature : 30℃
Detection : UV at 230 nm
Injection : 10 µL (0.1 mg/mL)

デュロキセチン塩酸塩（米国薬局方記載条件）
Duloxetine hydrochloride (The United States Pharmacopeia)

Duloxetine hydrochloride (The United States Pharmacopeia) F130930A

System suitability solution*

<table>
<thead>
<tr>
<th>System suitability requirement</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resolution (1, 2)</td>
<td>≥ 3.5</td>
</tr>
<tr>
<td>Tailing factor (Duloxetine)</td>
<td>0.8 ~ 1.5</td>
</tr>
<tr>
<td>Tailing factor (Duloxetine related compound A)</td>
<td>0.8 ~ 1.5</td>
</tr>
</tbody>
</table>

*System suitability solution was prepared from duloxetine hydrochloride and duloxetine related compound A supplied as reagents for laboratory use.

フェノプロフェン
Fenoprofen

Fenoprofen V150427B

Column : CHIRAL ART Amylose-SA (5 µm)
Eluent : n-hexane/2-propanol/TFA (90/10/0.1)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 254 nm
Injection : 10 µL (0.1 mg/mL)
**Hexobarbital (Flurbiprofen)**

Column: YMC CHIRAL NEA (R)  
250 x 4.6 mm I.D.  
Eluent: acetonitrile/water (30/70)  
Flow rate: 0.7 mL/min  
Temperature: ambient  
Detection: UV at 210 nm  
Injection: 1 µL (1.2 mg/mL)

**Hydroxychloroquine (Hydroxychloroquine)**

Column: CHIRAL ART Amylose-SA (5 µm)  
250 x 4.6 mm I.D.  
Eluent: n-hexane/2-propanol/diethylamine (90/10/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25°C  
Detection: UV at 254 nm  
Injection: 10 µL (0.1 mg/mL)

**Ibuprofen (Ibuprofen)**

Column: CHIRAL ART Amylose-SA (5 µm)  
250 x 4.6 mm I.D.  
Eluent: n-hexane/2-propanol/TFA (99/1/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25°C  
Detection: UV at 220 nm  
Injection: 10 µL (1 mg/mL)

**Hexobarbital (Hexobarbital)**

Column: YMC CHIRAL NEA (R)  
250 x 4.6 mm I.D.  
Eluent: acetonitrile/water (30/70)  
Flow rate: 0.7 mL/min  
Temperature: ambient  
Detection: UV at 210 nm  
Injection: 1 µL (1.2 mg/mL)
2,2'-イソプロピリデンビス(4-フェニル-2-オキサゾリン)  
2,2'‑Isopropylidenebis(4‑phenyl‑2‑oxazoline)  
T180515F

Column: YMC CHIRAL NEA (R)  
250 X 4.6 mm I.D.  
Eluent: acetonitrile/0.5 M NaClO₄ (40/60)  
Flow rate: 1.0 mL/min  
Temperature: ambient  
Detection: UV at 268 nm  
Injection: 10 µL (1.4 mg/mL)

ケトプロフェン  
Ketoprofen  
V150609K

Column: CHIRAL ART Cellulose-SC (5 µm)  
250 X 4.6 mm I.D.  
Eluent: n-hexane/2-propanol/TFA (90/10/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25°C  
Detection: UV at 254 nm  
Injection: 10 µL (0.1 mg/mL)

ケトロラック  
Ketrolac  
H170821D

Column: CHIRAL ART Cellulose-SJ (5 µm)  
250 X 4.6 mm I.D.  
Eluent: n-hexane/tetrahydrofuran/TFA (70/30/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25°C  
Detection: UV at 305 nm  
Injection: 5 µL (1.0 mg/mL)
**Lansoprazole**

Column: CHIRAL ART Amylose-SA (5 µm)  
Eluent: ethyl acetate/ethanol/diethylamine (95/5/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25℃  
Detection: UV at 290 nm  
Injection: 10 µL (100 µg/mL)

**DL-Mandelic acid**

Column: CHIRAL ART Amylose-C (5 µm)  
Eluent: n-hexane/2-propanol/TFA (80/20/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25℃  
Detection: UV at 254 nm  
Injection: 20 µL (1 mg/mL)

**Metoprolol**

Column: CHIRAL ART Cellulose-C (5 µm)  
Eluent: n-hexane/ethanol/ethanolamine (80/20/0.1)  
Flow rate: 1.0 mL/min  
Temperature: 25℃  
Detection: UV at 254 nm  
Injection: 5 µL (1 mg/mL)
**Metoprolol**

**Column**: CHIRAL ART Cellulose-C (5 µm)

**Eluent**: Methanol

**Flow rate**: 1.0 mL/min

**Temperature**: 25 °C

**Detection**: UV at 254 nm

**Injection**: 5 µL (1 mg/mL)

---

**Ofloxacin (Levofoxicin)**

**Column**: CHIRAL ART Cellulose-SC (5 µm)

**Eluent**: MTBE*/ethanol/acetic acid/ethylenediamine (50/50/0.1/0.1)

**Flow rate**: 1.0 mL/min

**Temperature**: 40 °C

**Detection**: UV at 300 nm

**Injection**: 5 µL (100 µg/mL)

* methyl tert-butyl ether

---

**Phenoxybenzamine**

**Column**: CHIRAL ART Cellulose-SJ (5 µm)

**Eluent**: Methanol

**Flow rate**: 1.0 mL/min

**Temperature**: 25 °C

**Detection**: UV at 270 nm

**Injection**: 5 µL (1 mg/mL)

---

**N-CBZ-DL-Phenylalanine**

**Column**: CHIRAL ART Cellulose-SC (5 µm)

**Eluent**: Methanol

**Flow rate**: 1.0 mL/min

**Temperature**: 40 °C

**Detection**: UV at 300 nm

**Injection**: 5 µL (100 µg/mL)

---

**Phenoxybenzamine**

**Column**: CHIRAL ART Cellulose-SJ (5 µm)

**Eluent**: Methanol

**Flow rate**: 1.0 mL/min

**Temperature**: 25 °C

**Detection**: UV at 270 nm

**Injection**: 5 µL (1 mg/mL)
**Pindolol**

**Column**: CHIRAL ART Cellulose-SB (5 µm)  
**Eluent**: methanol/diethylamine (100/0.1)  
**Flow rate**: 1.0 mL/min  
**Temperature**: 25°C  
**Detection**: UV at 265 nm  
**Injection**: 10 µL (100 µg/mL)

**Propranolol**

**Column**: CHIRAL ART Cellulose-C (5 µm)  
**Eluent**: n-hexane/2-propanol/diethylamine (80/20/0.1)  
**Flow rate**: 1.0 mL/min  
**Temperature**: 25°C  
**Detection**: UV at 230 nm  
**Injection**: 10 µL (0.1 mg/mL)

**Rabeprazole**

**Column**: CHIRAL ART Cellulose-SC (5 µm)  
**Eluent**: ethyl acetate/2-propanol/diethylamine (95/5/0.1)  
**Flow rate**: 1.0 mL/min  
**Temperature**: 25°C  
**Detection**: UV at 290 nm  
**Injection**: 5 µL (100 µg/mL)
Sertraline hydrochloride (The European Pharmacopoeia)

Column: CHIRAL ART Amylose-C (5 µm)
Eluent: mixture of n-hexane/2-propanol (90/10)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 275 nm
Injection: 20 µL

* Test solution and Reference solution were prepared from Sertraline hydrochloride supplied as a reagent for laboratory use.

** n-hexane/2-propanol/diethylamine (97/5/5)

Column: CHIRAL ART Amylose-C Neo (5 µm)
Eluent: n-hexane/2-propanol (90/10)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 230 nm
Injection: 5 µL (0.1 mg/mL)

Column: CHIRAL ART Cellulose-C (5 µm)
Eluent: n-hexane/2-propanol (90/10)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 230 nm
Injection: 5 µL (0.1 mg/mL)

Column: CHIRAL ART Cellulose-SJ (5 µm)
Eluent: n-hexane/tetrahydrofuran (90/10)
Flow rate: 1.0 mL/min
Temperature: 25°C
Detection: UV at 254 nm
Injection: 10 µL (0.1 mg/mL)
トリメブチン
Trimebutine

```
Column : CHIRAL ART Cellulose-SJ (5 µm)
250 X 4.6 mmI.D.
Eluent : n-hexane/ethanol/diethylamine
(95/5/0.1)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 265 nm
```

α : 1.43
Rs : 3.36

トレガー塩基
Troger’s base

```
Column : CHIRAL ART Amylose-SA (5 µm)
250 X 4.6 mmI.D.
Eluent : n-hexane/ethanol (90/10)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 254 nm
Injection : 10 µL (0.1 mg/mL)
```

α : 2.3
Rs : 12.2

バルサルタン（米国薬局方記載条件）
Valsartan (The United States Pharmacopeia)

```
Column : CHIRAL ART Cellulose-C (5 µm)
250 X 4.6 mmI.D.
Eluent : n-hexane/2-propanol/TFA (85/15/0.1)
Flow rate : 0.8 mL/min
Temperature : 25℃
Detection : UV at 230 nm
Injection : 10 µL
(The United States Pharmacopeia 34th; Related compounds)
```

α : 1.3
Rs : 4.2

ベラパミル
Verapamil

```
Column : CHIRAL ART Amylose-C (5 µm)
250 X 4.6 mmI.D.
Eluent : n-hexane/2-propanol/diethylamine
(90/10/0.1)
Flow rate : 1.0 mL/min
Temperature : 25℃
Detection : UV at 254 nm
Injection : 10 µL (1 mg/mL)
```

* Test solution was prepared from Valsartan supplied as a reagent for laboratory use.
**Warfarin**

![Warfarin structure](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CHIRAL ART Cellulose-SB (5 µm)</td>
</tr>
<tr>
<td>Eluent</td>
<td>acetonitrile/20 mM phosphoric acid (50/50)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.0 mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Detection</td>
<td>UV at 254 nm</td>
</tr>
<tr>
<td>Injection</td>
<td>2 µL (200 µg/mL)</td>
</tr>
</tbody>
</table>

**Zopiclone**

![Zopiclone structure](image)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>CHIRAL ART Cellulose-SC (3 µm)</td>
</tr>
<tr>
<td>Eluent</td>
<td>10 mM ammonium bicarbonate (pH 8.0 with aqueous ammonia)/acetonitrile (25/75)</td>
</tr>
<tr>
<td>Flow rate</td>
<td>0.2 mL/min</td>
</tr>
<tr>
<td>Temperature</td>
<td>25°C</td>
</tr>
<tr>
<td>Detection</td>
<td>ESI, positive</td>
</tr>
<tr>
<td>Injection</td>
<td>10 µL (Hair sample extracted by micropulverized extraction)</td>
</tr>
<tr>
<td>Instrument</td>
<td>LC) Ultimate™ 3000 liquid chromatograph (Thermo Fisher Scientific) HRMS) Q Exactive™ mass spectrometer (Thermo Fisher Scientific)</td>
</tr>
</tbody>
</table>

**Spiked hair (5.0 ng/mg each)**

**Extracted ion chromatograms (m/z 389.11 > 245.022)**

*Courtesy of H. Miyaguchi, National Research Institute of Police Science.*
