

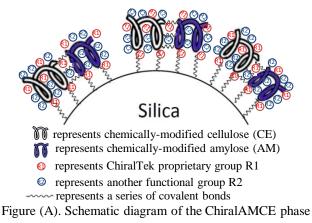
Sample Preparations

Short User Manual for ChiralAMCE Columns Please visit English website <u>http://chiraltek-column.com/Downloads.php</u> for downloading the latest full product manual and application notes for the ChiralAMCE columns.

All ChiralAMCE columns have been passed the quality control tests. Please kindly refer to the "Certificate of Quality Control Analysis" for information about the testing results. The column was stored in IPA/MeOH (50:50, v/v) before delivery. Please carefully read this user manual before using the column.

1. Unique Characteristics for ChiralAMCE columns

ChiralAMCE columns are the first type of chemicallymodified amylose-appended cellulose-immobilized silica particles-packed chiral columns (shown in Figure (A)). The ChiralAMCE particles were prepared through a specially-designed procedure by bonding different functional groups-substituted amylose-appended cellulose (AMCE) onto surface of high-quality porous silica (2 μ m, 3 μ m, 5 μ m, or 10 μ m). The column contains a unique complex chiral selector with two recognition moieties: amylose and cellulose. A single ChiralAMCE column can be used as two chiral columns: one amylose column and one cellulose-based column.



2. Application and Requirements

The ChiralAMCE columns can be used under multiple modes of mobile phase conditions. For use under reversed-phase conditions, the columns need to be firstly flushed with methanol following by mobile phase until reaching a constant column pressure. Similarly, for use under normal phase conditions, the columns need to be flushed with ethanol following by mobile phase until achieving a stable baseline signal. A ChiralAMCE or C18 guard column can be used under RP conditions and a ChiralAMCE or Diol guard column can be used for NP conditions. If non-standard mobile phases are to be used, please contact ChiralTek for technical support. Other manufacturers' columns contain a single type of chiral selector (e.g., amylose, or cellulose, etc). The ChiralAMCE column contains both amylose and cellulose. Figure (B) shows the schematic structure of the chemically-modified amylose-appended cellulose selector (B1) and the general glucose unit (B2) in ChiralAMCE column. Novel space structure with extra chiral recognition sites is formed between the amylose and cellulose moieties. Due to the cooperative functioning of the amylose and cellulose, the ChiralAMCE columns can provide different and generally better chiral separation abilities for a wider range of chiral compounds. @ (R1)

| (B1) | (B2) |
|----------------|--|
| M.C. | |
| ChiralAMCE-1: | R2= Phenylcarbamate; |
| ChiralAMCE-2: | R2= 3,5-Dimethylphenylcarbamate; |
| ChiralAMCE-3: | R2= 3-Chloro-4-methyl-phenylcarbamate; |
| ChiralAMCE-4: | R2= 3,5-Dichlorophenylcarbamate; |
| ChiralAMCE-5: | R2= 3-Chloro-5-methyl-phenylcarbamate; |
| ChiralAMCE-6: | R2= (S)-α-Methylbenzylcarbamate; |
| ChiralAMCE-7: | R2= 4-Methylbenzoate; |
| ChiralAMCE-8: | R2= 4-Methylphenylcarbamate; |
| ChiralAMCE-9: | R2= 4-Chloro-3-methyl-phenylcarbamate; |
| ChiralAMCE-10: | R2= 5-Chloro-2-methyl-phenylcarbamate; |
| | R2= 3-Chloro-2-methyl-phenylcarbamate; |
| ChiralAMCE-12: | R2= 4-Chlorophenylcarbamate. |
| | |

Figure (B). Schematic diagram of AMCE complex selector

When using ChiralAMCE columns with $2\mu m$ and $3\mu m$ particles, low flow rate (e.g., 0.1-0.3 mL/min) should be applied when used in traditional HPLC with highly viscous mobile phases in order to avoid high back pressure. However, there is no special flow rate limitation for use in UPLC.

| Flow direction: | Arrow direction on the label | |
|-----------------|-------------------------------------|--|
| Pressure: | < 860 bar (~12500 psi , 2 µm, 3 µm) | |
| | < 600 bar (~9000 psi, 5 µm, HPLC) | |
| Temperature: | | |
| Guard column: | ChiralAMCE, C18 or Diol column | |
| Mode: | HPLC, SFC, or UPLC | |

3. Care and Maintenance of the ChiralAMCE Columns

 It is strongly recommended to use ChiralAMCE, C18 or Diol guard columns to protect ChiralAMCE columns;
 It'd better to resolve samples in mobile phases and filter through 0.5µm membrane before injection;

[3]The solvent in the ChiralAMCE columns should be replaced with methanol (reversed phase conditions) or ethanol (normal phase conditions) if the columns need to be stored for over a week's time. [4] The ChiralAMCE columns can be easily cleaned by flushing with 100% methanol (reversed phase conditions) or 100% ethanol (normal phase conditions) at a proper flow rate for 3 hours.

[5]When worked in high pressure conditions, it's strongly recommended to gradually decrease flow rate to ensure column pressure lower than 100 bar (~1450 psi) before switching off the chromatograph pump.

4. Notice and Other Considerations

[1] A single ChiralAMCE column can be used under normal phase, reversed phase, or polar organic mobile phase conditions. It is strongly recommended to use 100% ethanol or IPA as intermediate solvent when switching between different mobile phase conditions. Due to the high viscosity of the IPA, low flow rate of about 0.1~0.3 mL/min should be applied in traditional HPLC in order to avoid extreme high pressure. However, there is no flow rate limit for UPLC.
[2]Diethylamine, butylamine, or amino ethyl alcohol (0.1%) can be used as mobile phase additives for basic compounds.
[3] Formic acid, acetic acid, or trifluoroacetic acid (0.1%) can be used as mobile phase additives for acidic compounds.
[4] Since the strong alkalic compounds (e.g., NaOH etc.) can cause damages to the ChiralAMCE column bed, they cannot be used as mobile phase additives or sample solution additives.

[5] All ChiralAMCE columns can be used in SFC under different type of mobile phase conditions.

5. List of the typical ChiralAMCE Columns with Different Specifications

| Product List of typical ChiralAMCE Columns from ChiralTek | | | |
|---|---------------|--------------------------|--|
| Part Number | Туре | Dimension | Description |
| 872-AMCE1-01 | ChiralAMCE-1 | 2 μm, 120Å, 50 × 2.1mm | 2 µm AMCE-1 bonded analytical column |
| 872-AMCE2-02 | ChiralAMCE-2 | 2 μm, 120Å, 100 × 2.1mm | 2 µm AMCE-2 bonded analytical column |
| 872-AMCE3-03 | ChiralAMCE-3 | 2 μm, 300Å, 150 × 2.1mm | 2 µm AMCE-3 bonded analytical column |
| 873-AMCE4-01 | ChiralAMCE-4 | 3 μm, 120Å, 50 × 2.1mm | 3 µm AMCE-4 bonded analytical column |
| 873-AMCE5-02 | ChiralAMCE-5 | 3 μm, 300Å, 100 × 2.1mm | 3 µm AMCE-5 bonded analytical column |
| 8573-AMCE6-03 | ChiralAMCE-6 | 3 μm, 500Å, 150 × 2.1mm | 3 µm AMCE-6 bonded analytical column |
| 8573-AMCE7-04 | ChiralAMCE-7 | 3 μm, 500Å, 200 × 2.1mm | 3 µm AMCE-7 bonded analytical column |
| 8573-AMCE8-05 | ChiralAMCE-8 | 3 μm, 500Å, 250 × 2.1mm | 3 µm AMCE-8 bonded analytical column |
| 873-AMCE9-01 | ChiralAMCE-9 | 3 μm, 120Å, 50 × 2.1mm | 3 µm AMCE-9 bonded analytical column |
| 873-AMCE10-02 | ChiralAMCE-10 | 3 μm, 120Å, 100 × 2.1mm | 3 µm AMCE-10 bonded analytical column |
| 873-AMCE11-03 | ChiralAMCE-11 | 3 μm, 120Å, 150 × 2.1mm | 3 µm AMCE-11 bonded analytical column |
| 8973-AMCE12-03 | ChiralAMCE-12 | 3 μm, 1000Å, 150 × 2.1mm | 3 µm AMCE-12 bonded analytical column |
| 8973-AMCE4-04 | ChiralAMCE-4 | 3 μm, 1000Å, 200 × 2.1mm | 3 µm AMCE-4 bonded analytical column |
| 8973-AMCE4-05 | ChiralAMCE-4 | 3 μm, 1000Å, 250 × 2.1mm | 3 µm AMCE-4 bonded analytical column |
| 8973-AMCE5-01 | ChiralAMCE-5 | 3 μm, 1000Å, 50 × 2.1mm | 3 µm AMCE-5 bonded analytical column |
| 8973-AMCE5-02 | ChiralAMCE-5 | 3 μm, 1000Å, 100 × 2.1mm | 3 µm AMCE-5 bonded analytical column |
| 8973-AMCE5-03 | ChiralAMCE-5 | 3 μm, 1000Å, 150 × 2.1mm | 3 µm AMCE-5 bonded analytical column |
| 8973-AMCE5-04 | ChiralAMCE-5 | 3 μm, 1000Å, 200 × 2.1mm | 3 µm AMCE-5 bonded analytical column |
| 8973-AMCE5-05 | ChiralAMCE-5 | 3 μm, 1000Å, 250 × 2.1mm | 3 µm AMCE-5 bonded analytical column |
| 8975-AMCE2-05 | ChiralAMCE-2 | 5 μm, 1000Å, 250 × 4.6mm | 5 µm AMCE-2 bonded analytical column |
| 8975-AMCE5-05 | ChiralAMCE-5 | 5 μm, 1000Å, 250 × 4.6mm | 5 µm AMCE-5 bonded analytical column |
| 833-SK1-03 | ChiralKit-1 | 3 μm, 1000Å, 150 × 2.1mm | Screening Kit-1 (3 analytical columns) |
| 833-SK2-03 | ChiralKit-2 | 3 μm, 1000Å, 150 × 2.1mm | Screening Kit-2 (6 analytical columns) |
| 705-AMCE2-13 | ChiralAMCE-2 | 5 μm, 150 × 10mm | 5 µm AMCE-2 semi-preparative column |
| 710-AMCE2-25 | ChiralAMCE-2 | 10 µm, 250 × 20mm | 10 µm AMCE-2 Preparative column |

ChiralAMCE columns with other dimensions are also available. This manual may not be updated on time, please visit English website <u>http://chiraltek-column.com/Downloads.php</u> for downloading the latest version of full product manual and application notes for ChiralAMCE columns. Please call an international phone number (+65)-93656129 to directly contact ChiralTek technical support team in Singapore. You also can call a special local phone number (+86)-95040358310 in the mainland of China to directly contact ChiralTek support team in Singapore.

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