**General Description**

Ultron ES-OVM-C is a column used for the separation of chiral isomers (enantiomeric compounds). Chiral isomers have the same general chemical structure but the two structures cannot be super-imposed on each other and cannot be separated by conventional HPLC columns. The Ultron ES-OVM-C column contains a packing material that has numerous chiral recognition sites, making it applicable to a wide range of enantiomeric compounds. This column recognizes hydrogen-bonding, polar, ionic, and hydrophobic sites, as well as the three-dimensional structure of sample molecules.

The Ultron ES-OVM-C particles are silica-based, nominally 5 µm in diameter with 120 Å pores. A chiral-recognition protein, ovomucoid, is chemically bonded to the silica support.

This Ultron-ES-OVM-C column has been tested to meet the required resolution requirements under the USB guidelines for the analysis of Clopidogrel. An example of the analysis is shown below, and the actual analysis is included with your Ultron ES-OVM-C column.

**Conditions**

- **Column:** Ultron ES-OVM-C
- **Column size:** 4.6 mm × 150 mm
- **Mobile phase:** 10 mM KH₂PO₄/CH₃CN = 75/25
- **Injection:** 10 µL

**Mobile Phase Considerations**

**pH Effects**

A typical relationship between mobile phase pH and solute capacity factors (k') is shown in Figure 1. The k' of basic compounds generally increases at higher pH while the k' of acidic compounds generally decreases at higher pH levels. Acidic compounds also have decreased retention at pH levels below 3.9, the isoelectric point for ovo-mucoid. Resolution generally increases with pH increase for basic compounds. For acidic compounds, resolution can generally be increased with pH decrease.
Organic Solvent

The retention of sample compounds can be varied by changing the type of organic solvent used in the mobile phase (that is, methanol, ethanol, or acetonitrile) as well as by the concentration of the organic component. The effect seen is generally similar to that observed in conventional reversed-phase HPLC (Figure 3).

Salt Concentration

The effect of salt concentration in the mobile phase on peak retention is shown in Figure 2. No effect is seen above 20 mM. When low salt concentrations (for example, 5 mM) are used, the k' of acidic compounds often greatly increases, whereas the k' values of basic compounds slightly decrease.

Other Effects

Column Temperature

The relationship between column temperature and peak resolution appears complex. While column efficiency improves at higher temperatures, the resolution of two loosely eluting peaks may be optimal at some intermediate temperature.

Sample Loading

Column efficiency decreases rapidly with increasing sample weight injected into the column. Thus, a low sample load per injection (for example, less than 5 µg) is recommended.

Sample Age

It is very important to pay attention to sample age with the Clotipogrel analysis. In the case where the desired resolution is not being met, consider a new sample, as sample degradation can lead to lower resolution.
Applications

Ultron ES-OVM-C columns separate a wide variety of chiral isomers. Specifically, the Ultron ES-OVM-C column is tested for the analysis of Clopidogrel. However, other examples include pharmaceuticals such as the β-blocking drugs alprenolol and bunitrolol, antihistamines (chlorpheniramine), and nonsteroidal anti-inflammatory drugs (ibuprofen). Other applications include agrochemicals and racemic amines and acidic compounds.

Column Care

Buffered mobile phases with pH ranges of 2 to 7.5 (3 to 7 preferred) and common organic water-miscible solvents (for example, acetonitrile, methanol, ethanol, and propanol) can be used safely with the Ultron ES-OVM-C column. Mobile phases with > 50% organic solvent should not be used. Guard columns are recommended for most applications to protect the analytical column against deleterious contaminants. Samples containing extraneous materials that will be highly retained on the column should be carefully avoided. Contaminated columns exhibiting poor peak shapes sometimes are conveniently restored by flushing with 20 to 40 column volumes of 50% acetonitrile/distilled water. Columns are preferably stored in a mobile phase of 10 to 20% acetonitrile/distilled water (after flushing out any previously used buffer) when not in use for long periods.

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