Ultron ES-Pepsin

Datasheet

General Description

Ultron ES-Pepsin columns are used for the separation of chiral isomers (enantiomeric compounds). Chiral isomers have identical chemical structures and cannot be separated by conventional HPLC columns. The Ultron ES-Pepsin column contains a packing material with specific chiral recognition that is applicable to a number of important chiral compounds. Since the isoelectric point is lower than pH 1.0, ES-Pepsin has a minus charge in mobile phases of pH 3.0-6.0. Therefore, ES-Pepsin best performs separations of basic compounds. This column is complementary to the more universally applicable Ultron ES-OVM column.
Ultron ES-Pepsin particles are silica-based, nominally 5 µm in diameter with 120 Å pores. A chiral-recognition protein, Pepsin, is chemically bonded to the silica support.

Safety Considerations

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of liquid chromatographic equipment should be aware of the toxicity or flammability of their mobile phases.
- Because of its small particle size, dry packings are respirable. Columns should only be opened in a well-ventilated area.

Operational Guidelines

- The direction of flow is marked on the column.
- Reverse flow through the column should be avoided, except to attempt removal of inlet pluggage.
- The allowable pH range of the mobile phase is 3.0 to 6.0.
- Ultron ES-Pepsin columns are compatible with water and many commonly used organic solvents (e.g., methanol, ethanol, and acetonitrile). Organic solvent concentrations greater than 10% are not recommended.
- Maximum recommended operating pressure is 150 bar (2200 psi). Maximum flow rate is 1.0 mL/min for 4.6 x 150 mm columns.
- Maximum operating temperature is 30°C.
- When the ES-Pepsin column is not in regular use, it should be cleaned thoroughly by purging with 10/90 ethanol/20mM phosphate buffer (pH 5.0) at a flow of 0.8 mL/min for one-half hour, followed by five minutes of 20 mM phosphate buffer (pH 5.0) containing 0.02% sodium azide. The column should then be stored in a refrigerator.

Mobile Phase Considerations

- pH Effects

Retention of basic compounds such as chlorpheniramine (Figure 1) and alprenolol increase with eluent pH from 3.0 to 6.0. Maximum enantioselectivity is achieved at pH 5.0 Similar chromatographic behavior is observed for other basic compounds. The separation of the isomers of alprenolol illustrates this point (Figure 2).

- Organic modifiers

Use of organic modifiers are more limited than with the ES-OVM column. Organic-modifier content should

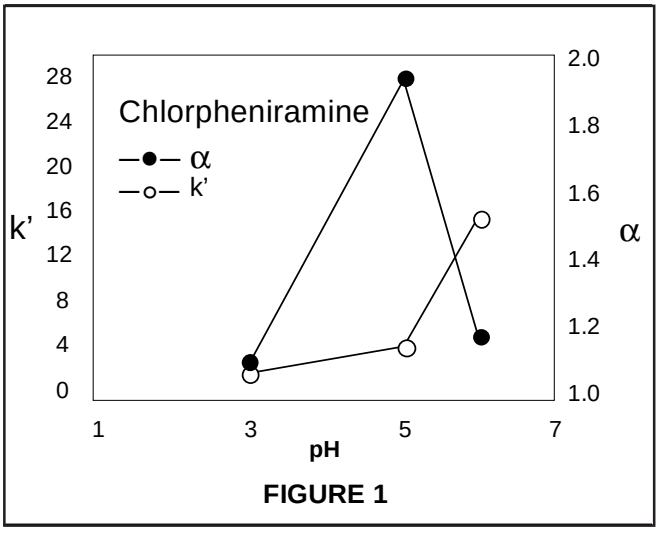

FIGURE 1

not exceed 10% of the mobile phase. In general, elution power increases in the order of methanol < ethanol < acetonitrile < 2-propanol. For most compounds tested, ethanol and acetonitrile provided better enantioselectivity.

Other Effects

• Column Temperature

Column efficiency generally improves with temperature. However, the use of elevated temperature is limited to 30°C. Resolution of closely eluting peaks can be optimal at temperatures below ambient for the ES-Pepsin column.

• Sample Loading

Column efficiency decreases rapidly with increasing sample weight injected into the column. A sample load ≤ 5 µg is recommended.

Applications

Ultron ES-Pepsin columns perform chiral separations of atenolol, salbutamol (Figure 3) and terbutaline, all of which are not obtained with the Ultron ES-OVM column. Chiral separations of alprenolol and bufetolol, which are not fully separated with ES-OVM, are well separated on ES-Pepsin. Since the range of separations is narrower for ES-Pepsin than ES-OVM, the two columns are best utilized as complementary pairs.

Ordering Information	Part No.
Ultron ES-Pepsin Column (5µ)	
4.6 mm ID x 150 mm	822111651
Guard Column	
4.0 mm ID x 10 mm	832111630

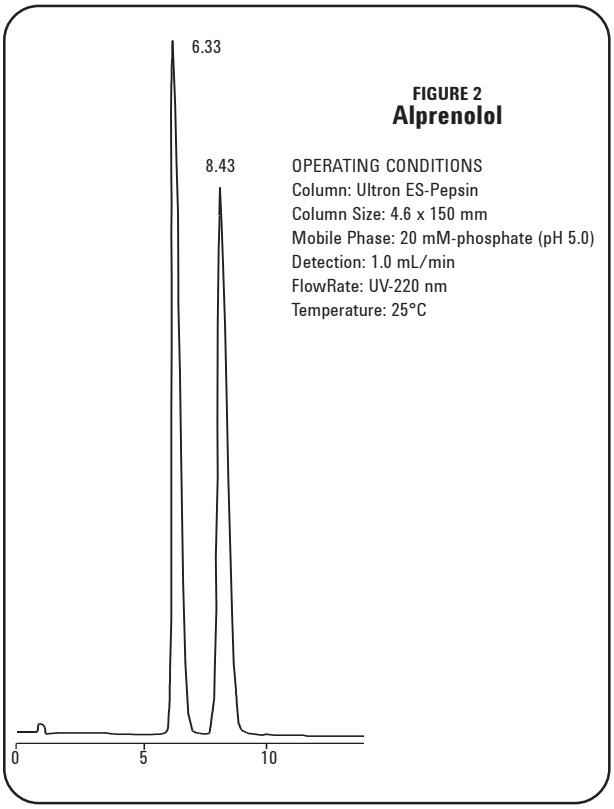

FIGURE 2
Alprenolol

OPERATING CONDITIONS
Column: Ultron ES-Pepsin
Column Size: 4.6 x 150 mm
Mobile Phase: 20 mM-phosphate (pH 5.0)
Detection: 1.0 mL/min
FlowRate: UV-220 nm
Temperature: 25°C

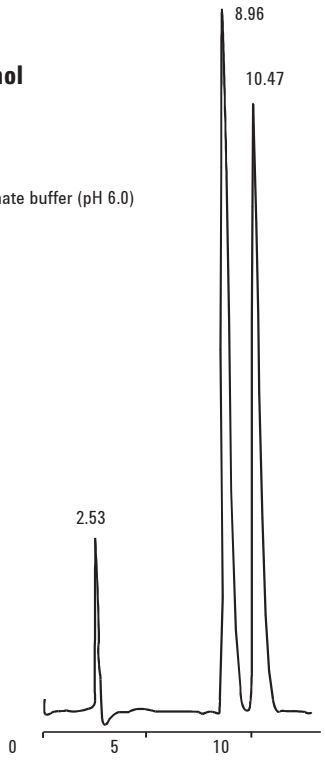

FIGURE 3
Salbutamol

OPERATING CONDITIONS
Column: Ultron ES-Pepsin
Column Size: 4.6 x 150 mm
Mobile Phase: 20 mM-phosphate buffer (pH 6.0)
Detection: 1.0 mL/min
Flow Rate: UV-220 nm
Termperature: 25°C

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