SHINWA CHEMICAL INDUSTRIES LTD.

ULTRON ES-OVM, ES-OVM-C

Chiral Chromatography Columns

The packing material used in these columns consists of a stable; highly denaturation resistant acidic glycoprotein (ovomucoid) covalently bonded to aminopropyl silica gel particles (5 µm). These columns are mainly designed for separations of the optical isomers of compounds consisting of aromatic rings possessing basic and acidic substituents under reversed phase analytical conditions.

Column dimensions (length x inner diameter (mm))	**150 x 2.0**	**150 x 4.6**	**150 x 6.0**
Applications	Semi-micro analytical column	Analytical column	Analytical column
Flow rate ranges	**Up to 0.2 mL/min**	**Up to 1.0 mL/min**	**Up to 1.5 mL/min**
Fittings	Waters compatible		
pH range	**Recommended range: 3.0～7.5**		
Organic solvent concentration range	**Recommended range: 1～10%** (Maximum allowable concentration: 50%)		
Analytical temperature range	**Recommended range: 0～30℃** (Maximum allowable temperature: 40℃)		
Analytical pressure range	**Recommended range: Up to 14.7 MPa** (Maximum allowable pressure: 19.6 MPa)		

Precautions when using these columns

- The same packing material is used for both the ULTRON ES-OVM and the ULTRON ES-OVM-C with the result that the performance for these columns is the same. The main difference between these columns is the fact that confirmation of the separation of benzoin is used for shipping checks for ES-OVM columns, while that for clopidogrel bisulfate and its related compounds are used for ES-OVM-C columns.
- ES-OVM columns are shipped containing 33% aqueous solutions of 2-propanol.
  ES-OVM-C columns are shipped containing 30% aqueous solutions of acetonitrile.
  When replacing these solutions with mobile phases with high salt concentrations or high viscosity levels, in order to avoid the danger of sudden pressure increases, when using 150 x 4.6 mm I.D. columns, flow the mobile phase at an initial flow rate of 0.2 ml/min, determine that the pressure is stable, and, determining that the pressure is stable at each step, increase the flow rate to the desired value in steps of 0.2 ml/min.
- Ensure to filter samples and mobile phases using a membrane filter with a mesh size of 0.45 µm or smaller before using these columns. Failure to filter mobile phases, etc. can lead to blockages of column filters and increases in analytical pressures.
- Ensure to thoroughly degas mobile phases prior to use. Insufficient degassing of mobile phases can lead to the formation of bubbles inside analytical instruments and columns resulting in problems with analyses.
- Although the packing material used in these columns consists of a highly denaturation resistant protein bound to silica gel particles, the maximum allowable organic solvent concentration under reversed phase conditions is 50%. However, the recommended organic

solvent concentration range of 1~10% is most effective in terms of separation performance. Excessively high concentrations of organic solvents can lead to the denaturation of the protein resulting in adverse effects with respect to analyses.

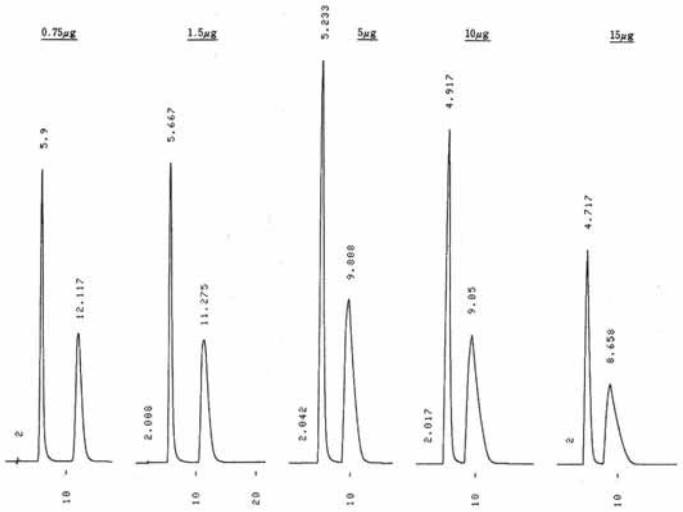
- The recommended analytical temperature range for these columns is 0~30℃. In addition, although the maximum operating temperature for these columns is 40℃, excessively high analytical temperatures can lead to the denaturation of the protein used in these columns resulting in adverse effects with respect to analyses.
- In order to extend column operating lifetimes as much as possible, we recommend using columns at analytical pressures up to 14.7 MPa. In addition, although the maximum allowable analytical pressure for these columns is 19.6 MPa, excessively high analytical pressures can lead to the formation of voids inside columns resulting in degradations in peak shapes.
- The pH range of mobile phases should be kept between 3.0 and 7.5. Excessively high levels of acidity can lead to detachment and denaturation of the protein used in these columns. In addition, excessively high levels of basicity can lead to dissolution of the silica gel used in these columns and denaturation of the protein.
- In order to avoid the build up of contamination such as scale and extend column lifetimes, we recommend the use of guard columns. The use of guard columns does not have any adverse effects on separation performance, etc.
- With regard to the equilibration of mobile phases, one of the separation modes of these columns is ionic interactions, with the result that a certain amount of time is required for mobile phase equilibration. In particular, it should be noted that equilibrations around the isoelectric point of ovomucoid (pI 4.1) used in the packing material for these columns require extended amounts of time.
  When changing the pH of a mobile phase from 3.0 to 4.6 for example, an extended amount of time will be required to thoroughly equilibrate the mobile phase.

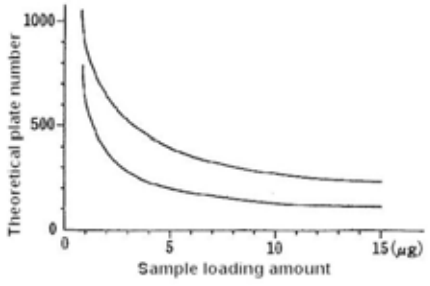
Optimizing analytical conditions

Sample loading amounts

Theoretical plate numbers decrease with increases in sample loading amounts. In order to achieve sharp peaks, we recommend keeping sample loading amounts as small as possible.

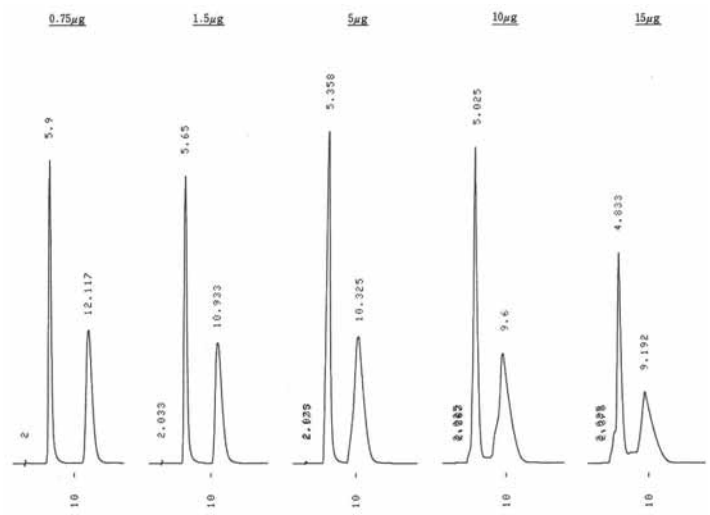
(1) Effects of sample loading weights

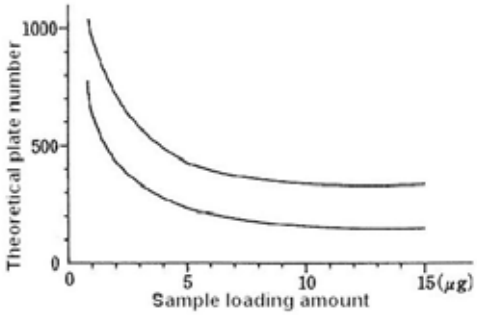




Column: **ULTRON ES-OVM** (4.6 mm ϕ × 150 mm)
Mobile phase: 20 mM KH_2PO_4 (pH = 5.5)/C_2H_5OH = 100/4
Flow rate: 1.0 mL/min.
Temperature: 25℃
Detection: UV-254 nm
Injection volume: 0.5 μL
Sample: Tolperison

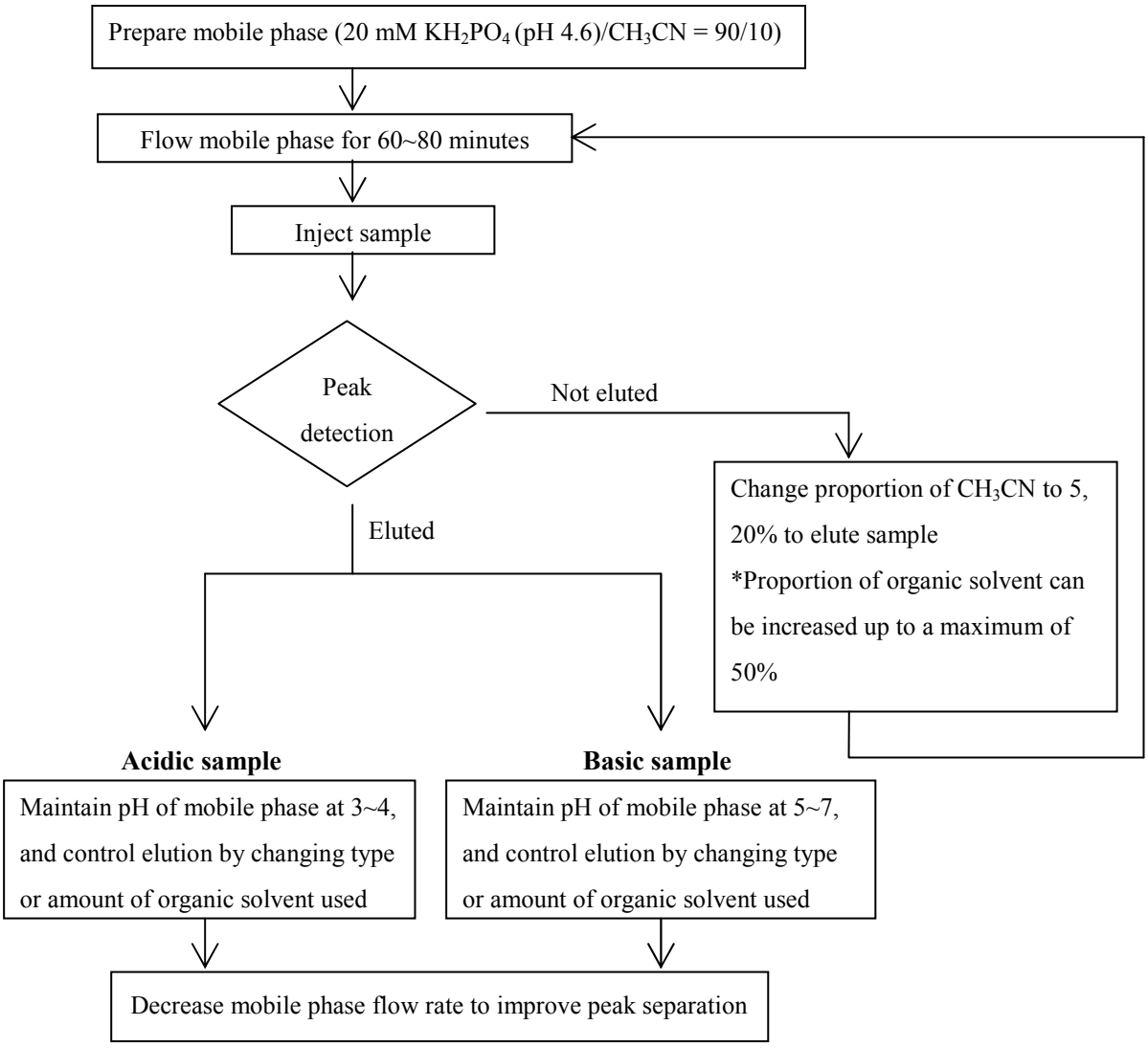
(2) Effects of sample loading volumes





Analytical conditions same as those shown in "Effects of sample loading weights" above.
Injection volumes: 0.5, 1.0, 3.3, 6.7, 10 μL

Flow diagram for optimization of mobile phase conditions



Effects of analytical temperature

With some exceptions, relatively good peak separation can be achieved by using analytical temperatures around 20℃. Shown below is a reference table showing the effects of analytical temperature on separations of propranolol.

	Column Temperature				
	5℃	11℃	19℃	28℃	40℃
k'1	18.67	11.49	7.79	4.97	2.59
k'2	23.94	14.59	9.68	6.05	3.06
α	1.28	1.27	1.25	1.22	1.18
*N*1	446	722	1059	1224	1899
*N*2	380	563	796	890	1282
Rs	1.12	1.35	1.44	1.21	1.08

Column: **ULTRON ES-OVM** (4.6 mm φ × 150 mm)
Mobile phase: 20 mM KH_2PO_4 (pH = 4.6)/C_2H_5OH = 100/10
Flow rate: 1.0 mL/min.
Detector: UV 220 nm (0.08 AUFS)
Sample: Propranolol

Cleaning and storage of columns

- When cleaning 150 x 4.6 mm I.D. columns, replace the buffer solution of the mobile phase used (e.g. 20 mM KH_2PO_4/acetonitrile = 90/10) with purified water, and then clean the column overnight using a solution with an organic solvent concentration the same as (water/acetonitrile = 90/10) or slightly higher than that of the mobile phase at a flow rate of 0.2 mL/min. When columns are contaminated, clean for at least 2 hours using an 80/20 solution of pH 3.5 phosphoric acid aqueous solution and 2-propanol at a flow rate of 0.2 mL/min.
- When not performing analyses for 2 or 3 days, replace the mobile phase inside the column with a 30% aqueous solution of acetonitrile, and store the column at around 25℃ (room temperature).

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