Regeneration of reversed phase HPLC columns

One of the most frequent questions we are asked concerns the best process for washing and regenerating HPLC columns.
Although it is often unsuccessful, here are some general procedures to follow to regenerate reverse phase columns:

1. Pass between 20 to 30 ml of 95: 5 water / acetonitrile (or methanol).
2. Then, make a 95: 5 to 5:95 water / acetonitrile (or methanol) gradient in about 10 minutes and hold this phase for about 1 hour.
3. Perform the reverse process and the column will now be in use.

Some notes:

4. If the column allows it (like most modern columns), you can perform the above process with the column inverted (don't forget to disconnect the detector!), to remove particles.
5. If hydrophobic contaminants are suspected, you can wash with successive gradients of acetonitrile (or methanol), isopropanol, tetrahydrofuran, dichloromethane and hexane, in that order, then perform the reverse process.

164 Avenue Joseph Kessel - Bâtiment 7 - 78960 Voisins le Bretonneux - France
Tél. : +33 9 72 32 10 17 - Fax : +33 1 83 64 60 45 - info@imchem.fr - www.imchem.fr