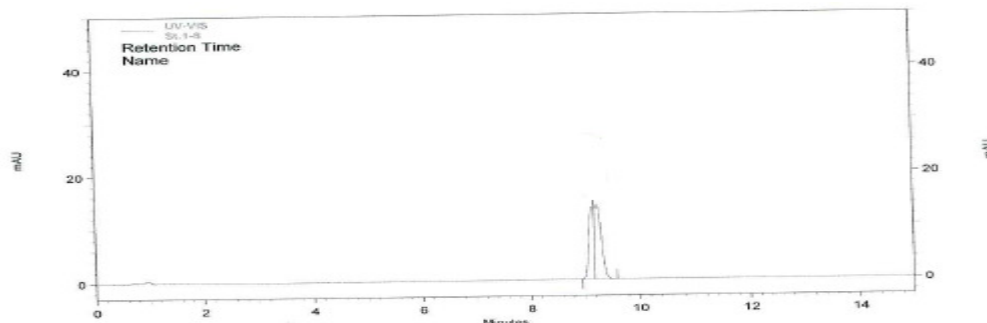




What can I do to remove split peaks?

Example:



Possible Causes:

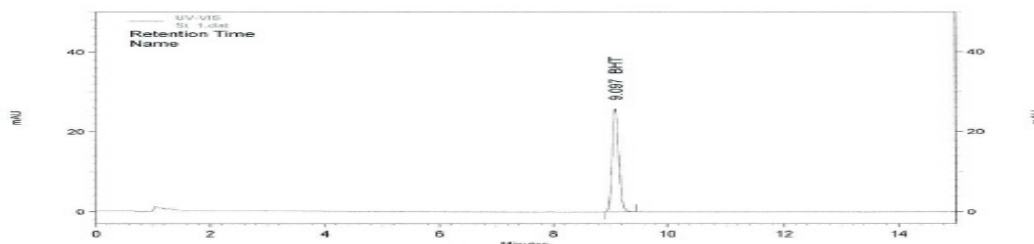
1. pH of the mobile phase is close to the pKa of a functional group on the compound and/or not buffered adequately.
2. a physical blockage obstructing the flow path
3. a void or channel in the column

Solutions:

1. Adjust the mobile phase pH +/- 2 units above or below the pKa of the compound's ionizable functional group, and buffer at that pH with an appropriate buffer (of sufficient buffer capacity to ensure the pH is controlled to the desired pH).
2. Preventative measures such as filtering the samples prior to injection and/or using a guard cartridge to protect the column are the best steps to take.
3. Discard column and replace with a new one (a void or channel in the column packing bed cannot be repaired or regenerated). If this occurred prematurely (expected column lifetime very application specific), consider the mobile phase components, backpressures, and sample to confirm if the running conditions are within the tolerances of the particular column.

Diagnosis:

In this case, the compound was butylated hydroxytoluene, with no ionizable functional groups. Column washes did not yield any improvements, however, reverse flushing the column quickly restored the peak shape (chromatogram below). This reversing of the column was therefore sufficient to remove the blockage on the column inlet that was obstructing the flow path (recommended to remove from detector to waste when reverse flushing to avoid contaminating the detector).



Key words: HPLC, split peaks, poor peak shape

Author: Scott Krepich