ChromaNik Technical Note 1002

The problem and its solution in the case of separation of amitriptyline in acidic, low-ionic-strength mobile phases

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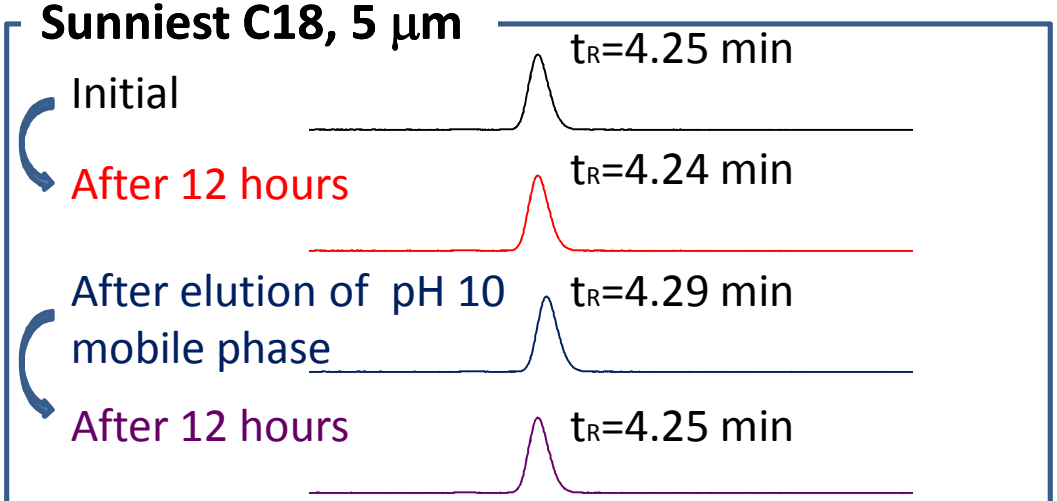
Problems in acidic, low-ionic strength condition

High purity silica C18 shows poor peak shape for analytical mass loads of basic analytes, and slow equilibration for basic analytes in acidic, low-ionic-strength mobile phases, when materials are exposed to alternating high and low pH mobile phases.

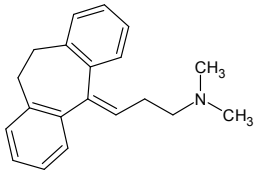
1. McCalley, D. V. *J. Chromatogr. A* ***2005, 1075, 57.***
2. (a) Gilroy, J. J.; Dolan, J. W.; Snyder, L. R. *J. Chromatogr. A* **2003, *1000, 757. (b) Marchand, D.H., Williams, L.A., Dolan,*** J.W., L.R. Snyder, L.R. *J. Chromatog. A,* ***2003, 1015, 53.***

Stability of retention under 0.1% formic acid mobile phase

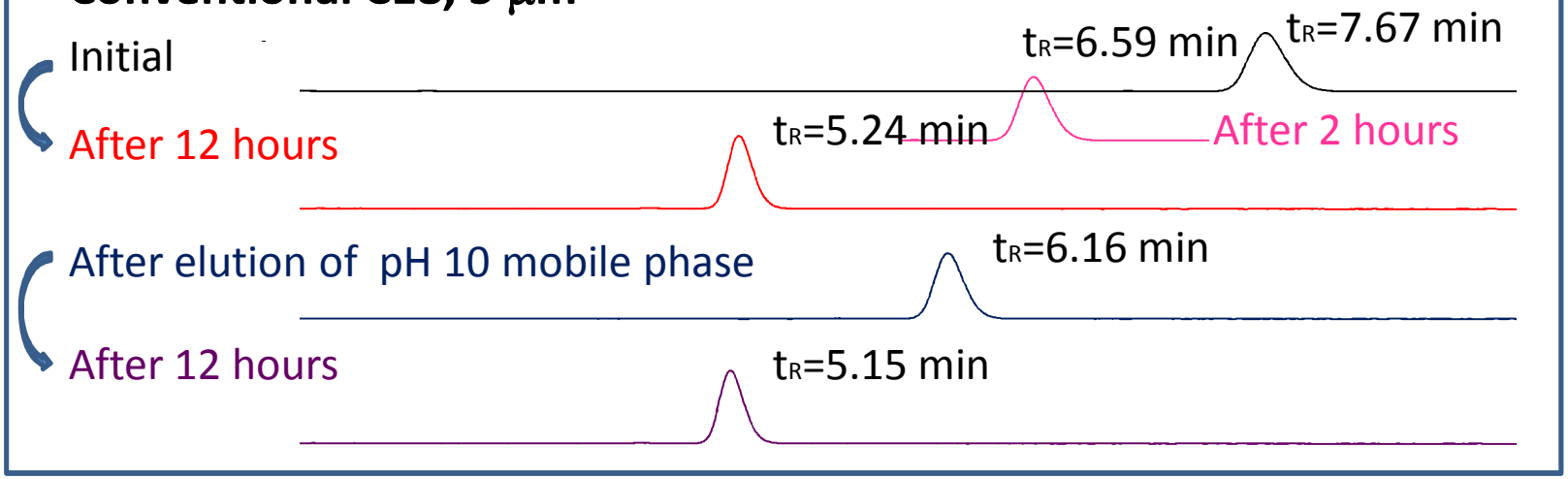
Sunniest C18, 5 μm



Condition
Column dimension: 150 x 4.6 mm
Mobile phase: Acetonitrile/0.1% formic acid (30:70)
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: Amitriptyline (0.27mg/mL)
Injection Volume:0.2 μL

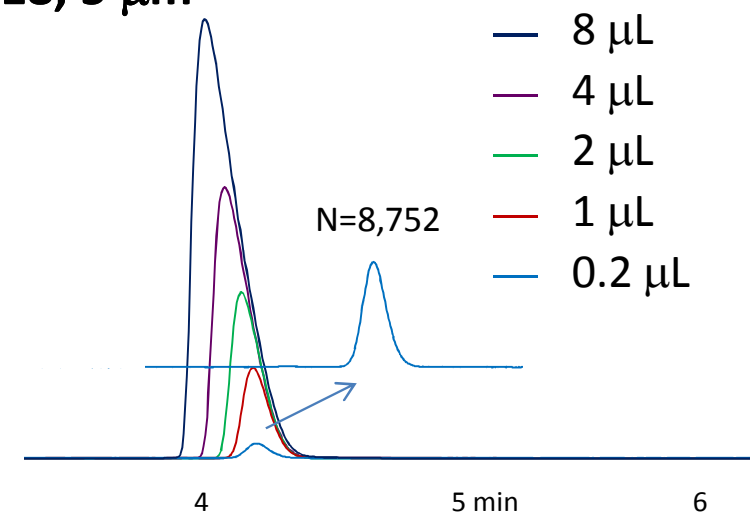


Conventional C18, 5 μm



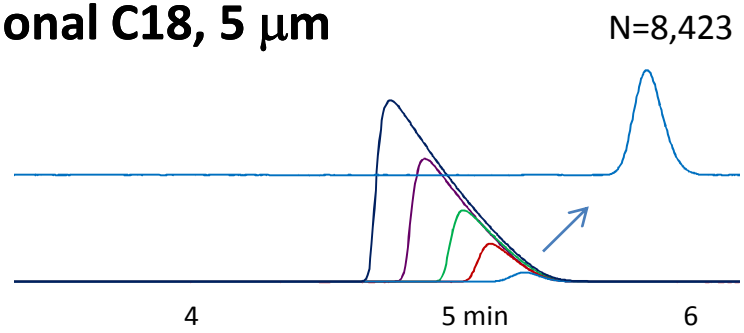
Loading capacity comparison of amitriptyline

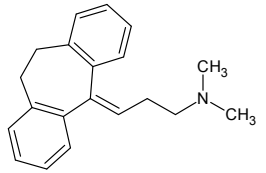
Sunniest C18, 5 μm



Condition
Column dimension: 150 x 4.6 mm
Mobile phase: Acetonitrile/0.1% formic acid (30:70)
Flow rate: 1.0 mL/min
Temperature: 40 ºC
Detection: UV@250nm
Sample: Amitriptyline (0.27mg/mL)
Injection Volume:0.2, 1, 2, 4 and 8 μL

Conventional C18, 5 μm





Only 5 ug of amitriptyline injection (0.2 μL) allows peak shape to be good for any RP-columns.

The solution of problem

Two PR columns such as Sunniest C18 and conventional C18 were compared with change of retention time and loading capacity of amitriptyline. Initial chromatograms were measured in one hour after mobile phase was changed. pH 10 mobile phase was acetonitrile/10 mM sodium carbonate, and flowed for one hour.
Sunniest C18 showed little change in retention for 12 hours, while conventional C18 indicated a large change in retention. Difference between these columns is an amount of residual silanol group in stationary phase. Sunniest C18 is much less residual silanol groups than most of conventional C18. Existence of residual silanol groups is the reason why change in retention occurs in acidic, low-ion-strength mobile phases.
Some company declared that charged surface technique was the solution for such a problem. It makes polarity of surface of a particle be high, so that silanol groups are solvated with water easily. It is considered that a silanol group solvated with water is more inert and occurs less unexpected change than an isolated silanol group around hydrophobic environment. However charged surface technique has a disadvantage that column life decreases.
Sunniest bonding technique is the solution without the sacrifice of column life.

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