

A Novel Bonding Technique Using a Polyfunctional Silyl-Reagent for Reversed-Phase Liquid Chromatography--- A NEW APPROCH !!!! II

Norikazu Nagae¹, Tomoyasu Tsukamoto¹, Anders Grahn², Ralf Jutvik² and Vinay D. Gaitonde²

1) ChromaNik Technologies Inc., 6-3-1 Namiyoko, Minato-ku, Osaka, Japan

2) Biotech AB, Box 133, 439 23, Onsala, Sweden



Abstract

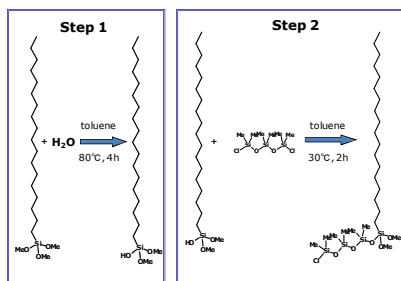
Reversed-phase LC columns and Sorbent development has experienced chromatographic performance improvement in terms of ULTRA pure silica, New End capping Reagents and chemistries, along with improvement in Bonding technology, innovative hybrid silica particle and high speed fused core Sorbents as well as sub 2 micron high efficiency sorbents and columns. These new innovative contributions are well recognized and accepted in HPLC work practices.

In regards to Bonding chemistries, most of reversed phase silica materials are monomerically or polymerically bonded with alkyl chain, then end-capped with trimethylsilane or hexamethyltrisiloxane etc. In this study, polyfunctional silyl-reagent was synthesized with octadecyltrimethoxysilane and hexamethyldichlorotrisiloxane. This reagent is called hexamethyloctadecyltetrasiloxane (HMOTDS - C18). We are able to bond the Chromatographic Silica particles with this reagent and finally end-cap with trimethylchlorosilane.

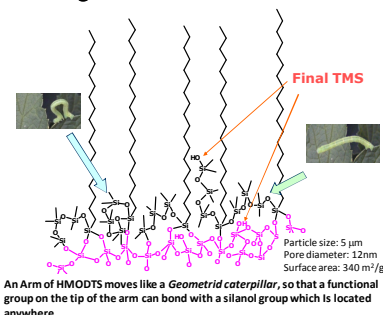
The resultant Sorbent was evaluated for chromatographic performance for resolving acidic and basic compounds in various Mobile phase compositions and Buffers. Stability of this phase was evaluated under both acidic and basic pH at high temperature. This phase showed symmetrical peaks of both acidic and basic compounds such as Formic acid and Amitriptyline. Interestingly enough we observed a symmetric peak of Amitriptyline in Mobile phase consisting of Acetonitrile and Ammonium acetate compositions. Majority of the C18 columns showed a long tailing peak of Amitriptyline under similar conditions. We observed that the Column life was more than 500 hours from pH 1.5 to pH 10 at 50 degree Celsius. A novel bonding technique using a polyfunctional silyl-reagent(HMOTDS - C18) could make effect of residual silanol groups the least and hence offer a tailing free chromatographic performance as well as endurance at elevated temperatures.

Novel C18 silyl-reagent (HMOTDS)

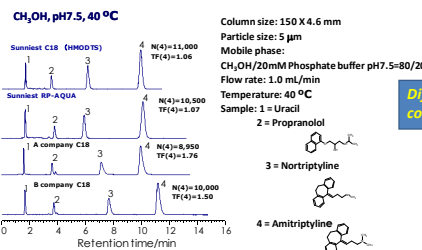
Patent pending



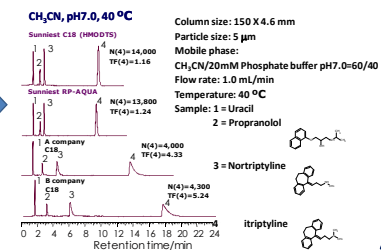
Bonding state of HMOTDS on silica



Evaluation of end-capping Comparison of amitriptyline peak I



Evaluation of end-capping Comparison of amitriptyline peak II



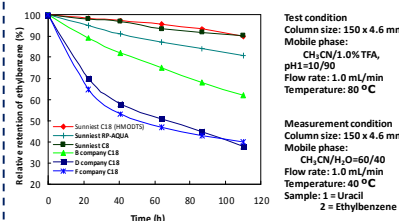
Different condition

Evaluation of end-capping Comparison of amitriptyline peak II-B

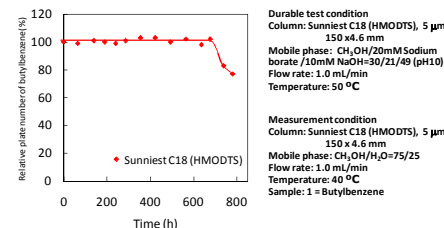
Column	TP (%)	Column	TP (%)
Sunniest C18 (HMOTDS)	1.24	Daicel ODS-ODS	2.34
Sunniest RP-AQUA	1.22	Sumi-NK C18	1.89
Acclaim 120 C18	5.19	Wako Purechem STAR RP-30s	2.60
Acclaim 120 C18	2.19	Inertsil ODS-5	1.30
ACE C18	3.25	Inertsil ODS-4	2.02
Ascentis C18	1.74	Inertsil ODS-3	2.70
Ascentis T3 C18	1.97	LC-column ODS	1.56
BioPhen C18	1.59	Shim-pack VP-ODS	3.44
Chromat C18	1.33	TAQAL ODS-100V	1.71
Columna SCD-C18	3.07	TAQAL ODS-100Z	2.71
Ultimate US-C18	2.52	Wakochem Navi C18-S	1.11
CAPCELLPAK C18 MG III	2.01	TMAC-Pack Pro C18	3.77
CAPCELLPAK C18 MG III	7.75	COMBAX Endcap Plus C18	3.28

Column size: 150 X 4.6 mm
Particle size: 5 µm
Mobile phase: CH₃CN/20mM Phosphate buffer pH7.0=60/40
Flow rate: 1.0 ml/min
Temperature: 40 °C
Sample: Amitriptyline

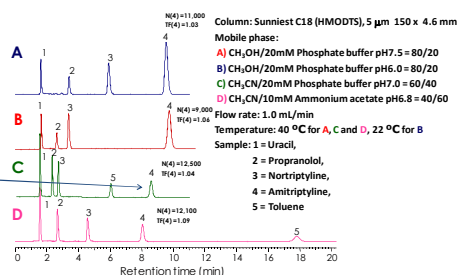
Stability under acidic condition



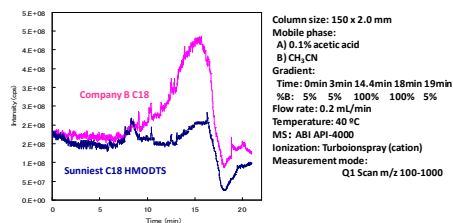
Stability under basic pH condition at 50 °C



Comparison of 4 kinds of mobile phase



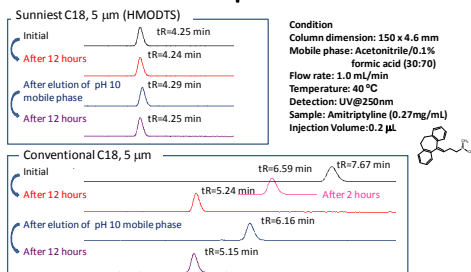
Bleed test using LC/MS



Conclusion

- ✓ Polyfunctional silyl-reagents were developed using C18 silyl reagent and end-capping reagent such as octadecyltrimethoxysilane, hexamethyldichlorotrisiloxane(HMOTDS).
- ✓ Functional group of HMOTDS can bond with any silanol groups on silica surface.
- ✓ There is the least effect of residual silanol groups on proposed C18 stationary phase. And basic compounds can be separated well without any restriction concerned with a mobile phase.
- ✓ Stable retention can be obtained by proposed C18 in acidic, low-ionic-strength mobile phases such as a mixture of 0.1% formic acid and acetonitrile. Furthermore under a such condition less tailing peaks was obtained.

Stability of retention under 0.1% formic acid mobile phase



Loading capacity comparison of amitriptyline

