

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

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## Abstract

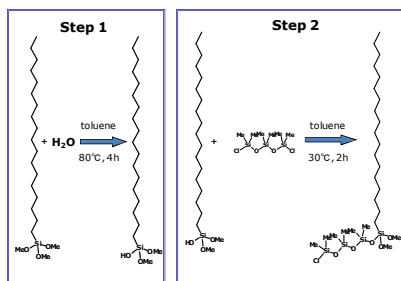
Reversed-phase LC columns and Sorbent development has experienced chromatographic performance improvement in terms of ULTRA pure silica, New End capping Reagents and chemistries, along with improvement in Bonding technology, innovative hybrid silica particle and high speed fused core Sorbents as well 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.

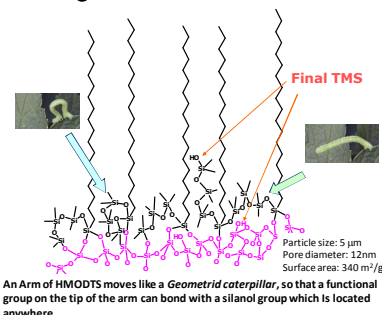
The resultant Sorbent was evaluated for chromatographic performance for resolving acidic and basic compounds in various Mobile phase compositions and Buffers. Stability of this phase was evaluated under both acidic and basic pH at high temperature. This phase showed symmetrical peaks of both acidic and 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)

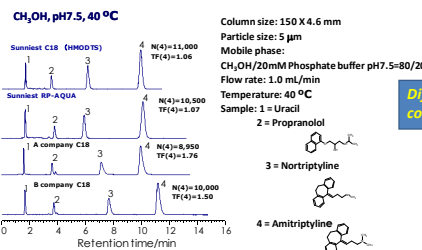
Patent pending



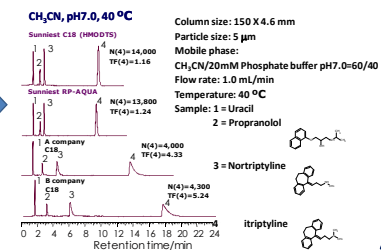
## Bonding state of HMOTDS on silica



## Evaluation of end-capping Comparison of amitriptyline peak I



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



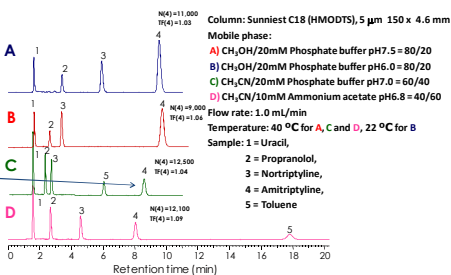
Different condition

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

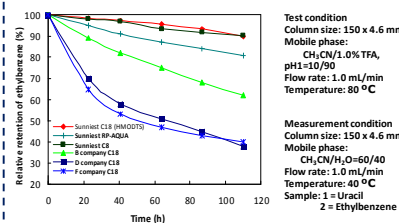
Column	TP	T <sub>R</sub>	Column	TP	T <sub>R</sub>
Sunnist C18 (HMOTDS)	1.24	3.700	Sunnist ODS-100S	2.34	6.700
Sunnist RP-AQUA	1.22	3.840	Sunnist-NK C18	1.89	6.100
Acclaim 120 Advantage II	5.19	3.500	Merck Puraguard STAR RP-18e	2.60	12.100
Acclaim 120 C18	2.19	3.400	Intertek ODS-5	1.30	12.000
ACE C18	3.25	5.300	Intertek ODS-P	2.02	8.000
Ascentis C18	1.74	6.300	Intertek ODS-3	2.70	6.100
Ascentis T3 C18	1.97	10.600	C-column ODS	1.56	10.600
BioPhen C18	1.59	10.100	Shim-pack VP-ODS	3.44	6.700
Chromat C18	1.33	10.000	TSQgel ODS-100V	1.71	10.000
ColuSense SCD-C18	3.07	8.100	TSQgel ODS-100Z	2.71	10.000
Ultimate US-C18	2.52	9.200	Wakoapak Navi C18-S	1.11	21.000
CAPCELLPAK C18 MG III	2.01	10.800	TMCC-Pack Pro C18	3.77	7.400
CAPCELLPAK C18 MG III	7.75	3.600	COMBAX Endura Plus C18	3.28	5.900

Column size: 150 X 4.6 mm  
Particle size: 5 μm  
Mobile phase: CH<sub>3</sub>CN/20mM Phosphate buffer pH7.0=60/40  
Flow rate: 1.0 ml/min  
Temperature: 40 °C  
Sample: Amitriptyline

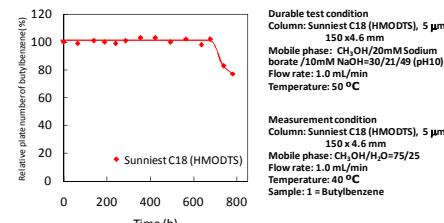
## Comparison of 4 kinds of mobile phase



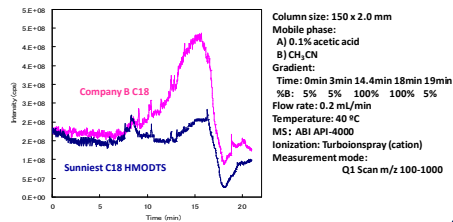
## Stability under acidic condition



## Stability under basic pH condition at 50 °C



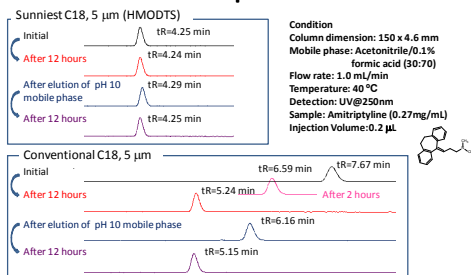
## Bleed test using LC/MS



## 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

