ASMS 2003

Study of Bonded Phases Cleaved from Silica-Based Liquid Chromatographic Packing Materials by ESI-MS

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Overview

In this work, we have studied the bonded phases cleaved from silica-based liquid chromatographic packing materials by electrospray ionization-mass spectrometry (ESI-MS). The detection sensitivities for mono, di, and trifunctional silanols, the hydrolysis products of the bonded phases, have been compared in positive and negative ion modes under acidic and basic conditions. The influence of chain length on the detection sensitivity have been evaluated with monofunctional silanols having chain length from one to eighteen carbons. The affects of polar groups on detection sensitivity have been tested with 15 polar compounds.

The ion species detected in positive and negative ion modes from the silanols and the polar compounds have been identified. The chloride adducts to the silanols have been observed and confirmed.

Introduction

Liquid chromatography/electrospray ionization-mass spectrometry (LC/ESI-MS) has become an important tool in proteomics, genomics, and drug metabolism and pharmacokinetics. However, it has been noticed that bleeding from liquid chromatographic packing materials may interfere with the characterization of analytes and reduce detection sensitivity. The bleeding substances can be cleaved bonded phases and residual reagents from silica-based materials, or degradation products, residual reagents and small oligomers from polymeric materials. Although ESI-MS has been used to analyze metal ions, inorganic and organic acids, and organic bases, few reports have been found on the characterization of organic silanols, the hydrolysis products of organic silyl ligands bonded on silica particles. In our work, we have focused on the understanding of the behavior of bonded phases cleaved from the silica-based materials under acidic and basic conditions.

Experimental

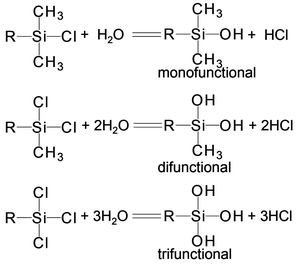
ESI-MS Conditions:

- Waters ZQ electrospray ionization-mass spectrometer
- Ionization mode: ES^+ or ES^-
- Capillary Volt.: 3.5 kV
- Cone Volt.: optimized for each ion of interest
- Source Block Temp.: 100°C ♦ Desolvation Temp.: 150°C
- Desolvation Gas Flow: 250L/h ♦ Cone Gas Flow: 50L/h
- Data Duration: 1 min.
- Data Mode: Collected in Continuum and converted to centroid (80%, Area) for calculations
- Infusion rate: 20 µL/min.

Preparation of Silanol Solutions:

- Dissolve mono, di, and trichlorosilanes in CH_2Cl_2
- Dilute with 90/10 (v/v) ACN/water to 10mM*-300mM (*A conc. low enough to eliminate the condensation for C18 silanols and high enough to neglect the electrolysis affect[1])
- Acidify/basify to 0.1% TFA/0.2% TEA as needed

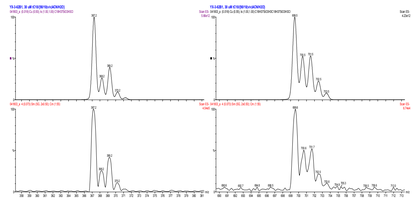
Reactions in Silanol Preparation:



R: CH_3, C_2H_5, C_4H_9, C_8H_{17} and $C_{18}H_{37}$ (C1-C4: monofunctional only)

Results and Discussion

Chloride Adducts to Silanols Found in ES^- Mode:



Observed spectra (bottom) and theoretical isotope patterns (top) for chloride adducts of $[M+Cl]^-$ (left) and $[2M+Cl]^-$ (right), where M is tC18 silanol molecule with MW = 332.

Further Confirmation on the Chloride Adducts in ES^- Mode:

Relative Intensity of $[M+Cl]^-$:

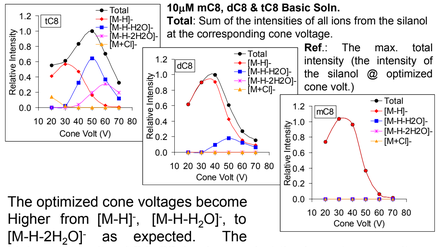
100µM	C8			C18		
Soln.	mono	di	tri	mono	di	tri
A (ref.)	-	1.0	1.0	1.0	1.0	1.0
B	-	0.5	0.5	0.4	0.4	0.3
C	-	-	-	-	-	-

10µM	C8			C18		
Soln.	mono	di	tri	mono	di	tri
A´(ref.)	-	1.0	1.0	1.0	1.0	1.0
B´	-	-	0.2	-	0.2	0.2
C´	-	-	-	-	-	-

Soln. A: 100µM mC8, dC8 & tC8 (or mC18, dC18 & tC18) with 0.6mM HCl
Soln. A´: 10µM mC8, dC8 & tC8 (or mC18, dC18 & tC18) with 0.06mM HCl
Soln. B/B´: Soln. A/A´ basified to 0.2% (14mM) TEA
Soln. C/C´: Soln. A/A´ acidified to 0.1% (14mM) TFA

The intensity of $[M+Cl]^-$ ions decreases with the addition of 0.2% TEA (Soln. B & B´) due to the deprotonation of silanols, and the adducts disappear in 0.1% TFA (Soln. C & C´) due to the complete protonation of silanols.
Detection sensitivity of the chloride adducts: tri > di > mono; and C18 > C8.

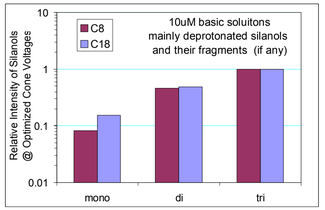
Ions Detected from Silanols in ES^- Mode:



10µM mC8, dC8 & tC8 Basic Soln.
Total: Sum of the intensities of all ions from the silanol at the corresponding cone voltage.
Ref.: The max. total intensity (the intensity of the silanol @ optimized cone volt.)

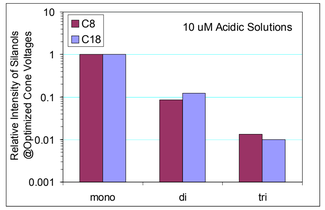
The optimized cone voltages become Higher from $[M-H]^-$, $[M-H-H_2O]^-$, to $[M-H-2H_2O]^-$ as expected. The highest intensity of $[M+Cl]^-$ is observed at the lowest cone voltage of 20V, i.e., the interactions between silanols and Cl^- are very weak. The same observation from C18 silanols.

Comparison of ES^- Detection Sensitivity among Mono, Di, and Tri-Functional Silanols:



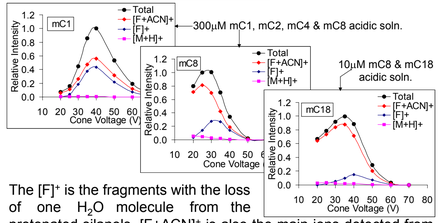
The sensitivity increases from mono, di, to trifunctional silanols. The acidity of silanols (proton-donors) increases in this order due to inductive effect.[2]

Comparison of ES^+ Detection Sensitivity among Mono, Di, and Trifunctional Silanols:



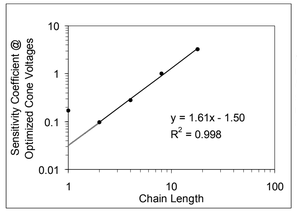
The sensitivity increases with the basicity of silanols (proton-acceptors): tri < di < monofunctional.

Ions Detected from Silanols in ES^+ Mode:



The $[F]^+$ is the fragments with the loss of one H_2O molecule from the protonated silanols. $[F+ACN]^+$ is also the main ions detected from di and trifuntional C8 and C18 silanols. The ACN adducts cannot be eliminated by increasing cone gas flow, but the intensity of $[F+ACN]^+$ relative to $[F]+$ decreases with silanol conc. since less ACN molecules surround each $[F]^+$ ion in the droplet.

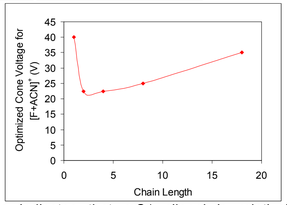
Influence of Chain Length on ES^+ Detection Sensitivity:



from 300µM mC1, mC2, mC4 & mC8 acidic soln and 10µM mC8 & mC18 acidic soln.

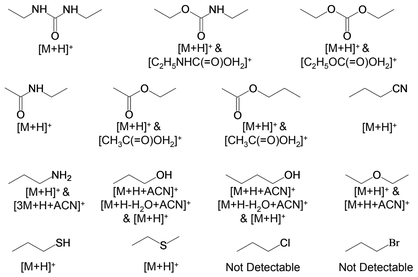
An excellent linear correlation in logarithmic scales between the chain length (mC2-mC18) and the sensitivity coefficient[1] (defined here as relative intensity of a silanol to mC8 at the same concentration). The increase of sensitivity with chain length can be due to surface-activity effects.[3]

Relatively Strong Solvation of mC1 Silanol:

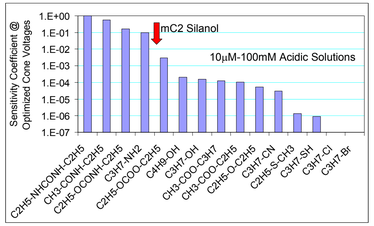


This Figure indicates that mC1 silanol is relatively strongly solvated. The transfer energy of a solvated ion from a solution to the gas phase is relatively low, and thus the rate constant for emission of such an ion from the droplet is relatively fast,[4] which could be why the sensitivity coefficient is relatively high for mC1 silanol as seen in the previous Figure.

Ions Detected in ES^+ for 15 Polar Compounds:



Comparison of ES^+ Detection Sensitivity among Polar Compounds and to mC2 Silanol:



Sensitivity coefficient is defined here as the relative intensity of a polar compound to 1,3-diethylurea normalized by their concentrations.

Conclusions

- The ES^- sensitivity increases with the acidity of silanols: mono < di < trifunctional. The ES^+ sensitivity increases with the basicity of silanols: tri < di < monofunctional.
- The sensitivity coefficient is linearly correlated with the chain length (mC2-mC18) in logarithmic scales. The relatively high sensitivity for mC1 silanol could be due to ion solvation.
- Urea, amide, carbamate and amine will be the main contributors to ES^+ bleeding from monofunctional bonded phases.
- Chloride adducts to silanols have been detected and confirmed. The ion species detected from the silanols and the polar compounds in ES^+ and/or ES^- modes have been identified.

References

1. Cole, R.B., Ed., *Electrospray Ionization Mass Spectrometry*; John Wiley: NY, 1997, Chap.1 & 3.
2. Streitwieser, A. Jr.; Heathcock, C.H., *Introduction to Organic Chemistry:* Macmillan: NY, 1981, p.240.
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