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At-Column-Dilution^(*) for Preparative Chromatography

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(*) Patent pending

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Abstract

Sample loading onto a column can be highly compromised due to the solubility of the compounds or their compatibility with the initial mobile phase conditions, resulting in low yields and productivity. Consequently, exploring alternative ways to load sample onto the column is urgently needed. The at-column-dilution method is a significant alternative as it allows an increase in sample loading, improving peak shapes, providing higher yield and productivity of the targeted compound. Case studies are shown where the benefits of the at-column-dilution method are demonstrated when loading basic compounds under acidic conditions and eluting at high pH, as well as when loading samples dissolved in strong sample solvents such as DMSO. The cases shown here illustrate the utility of the at-column-dilution technique while maintaining present day isolation and purification needs both in combinatorial chemistry as well as drug development.

Motivation for this Work

- Sample solvents can affect dramatically the chromatographic performance due to:
 - *Poor solubility of the sample in the loading solvent*
 - Limiting loading
 - Increasing injection volume
 - Reducing the number of samples processed daily
 - *Strong sample solvent effects*
 - Shifting retention times
 - Producing distorted peaks
- Increased costs and handling times occur if solvents have to be change before loading samples into the column
- A solution to this problem is urgently needed to purify samples dissolved under conditions not compatible with common initial mobile phase conditions

What is the At-Column-Dilution^(*) Method and Why Employ this Technique?

- The at-column-dilution technique permits the loading of sample onto the column parallel to the mobile phase stream.
- By employing this technique:
 - The risk of sample precipitation in the injector, loop or head of the column is eliminated
 - Sample loading increases drastically
 - Injection volume can decrease
 - Productivity for a given compound increases as fewer number and shorter runs can be readily accomplished
 - Retention shifts due to strong solvent effects are minimized
 - Peak shape improvement occurs

(*) Patent Pending

Loading Basic Compounds at High pH as the HCl Salt

- Hydrochloride salts are best dissolved in water for maximum solubility
- However, loading a base in the ionized form at high pH decreases the loadability
- High buffer concentrations in the gradient create solubility problems and impede MS detection
- At-column dilution into a high buffer concentration at the beginning of the gradient solves the problem

Loading Basic Compounds at High pH as the HCl Salt

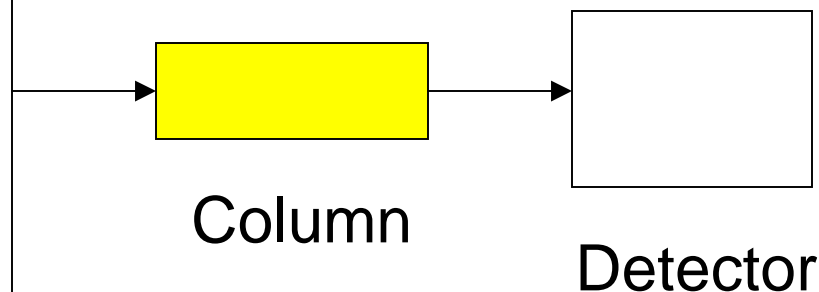
Diagram of At-Column-Dilution^(*)

water with a low buffer

- concentration feeds the sample

Gradient starts at a high buffer
concentration to load sample

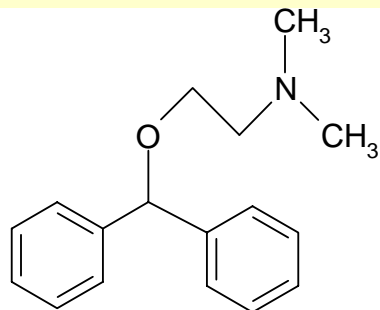
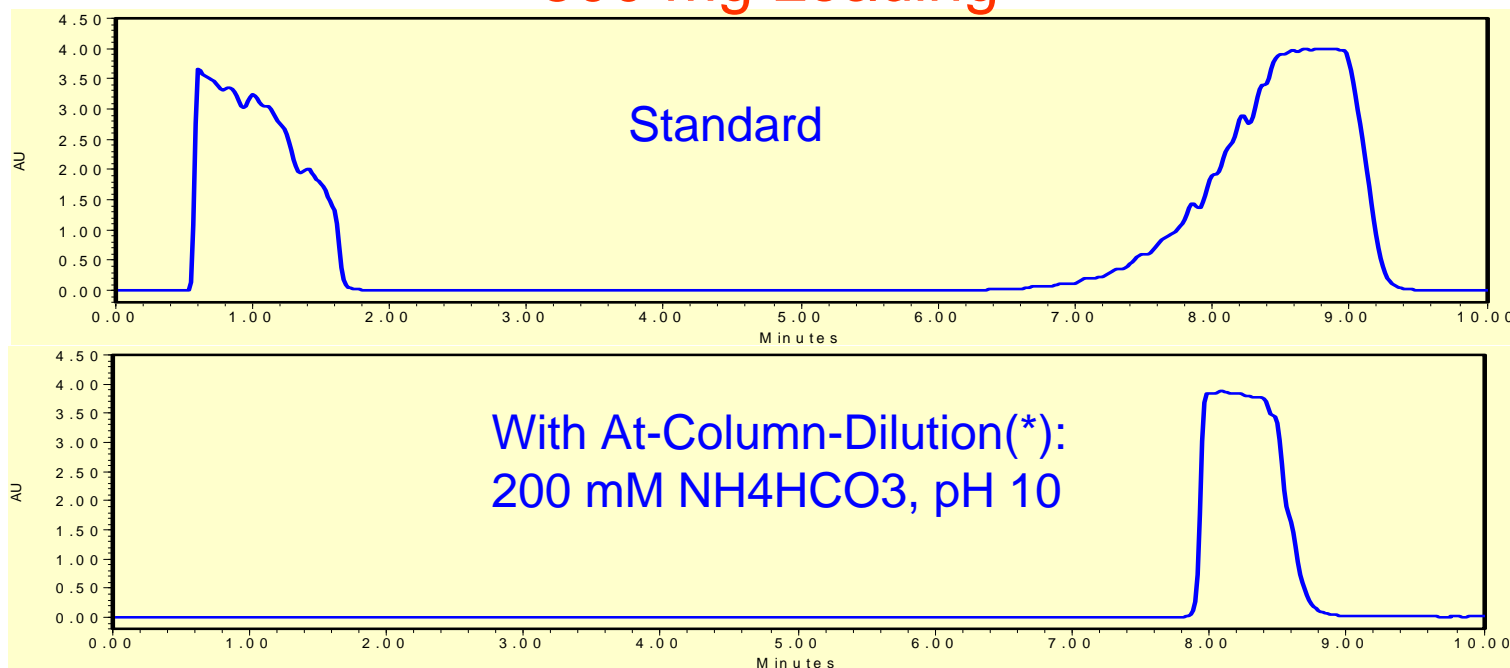
- onto column in a non-ionic
form at the beginning of the
gradient



^(*) Patent Pending

Loadability Comparison at High pH

800 mg Loading



Diphenhydramine

Conditions:

Column:

XTerra® Prep MS C18 19 x 50 mm, 5µm

Gradient:

Equilibrated for 5 min at 5% ACN,
then gradient $t_g = 5$ min, 5 to 90% ACN,
and hold at 90% ACN for 1 min,
The mobile phases contain 10 mM NH₄HCO₃, pH 10.0

Flow Rate:

30 mL/min

Analyte:

Diphenhydramine (800 mg) dissolved in H₂O

Injection Vol.:

2 mL

Detection:

254 nm

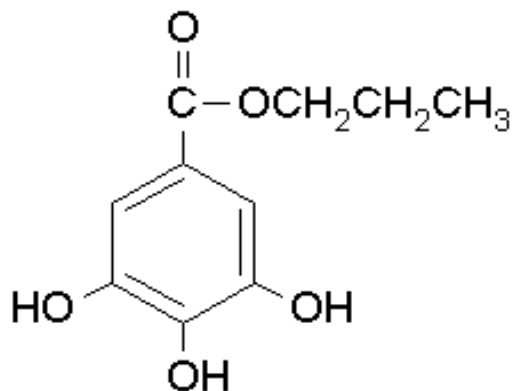
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Loading Samples Dissolved in Organic Solvents

- A substantial amount of samples are dissolved in organic solvents to increase solubility. However, under common initial mobile phase conditions, there is a high risk of precipitation within the injector, the loop and head of the column
- High viscosity solvents generate pressure spikes as the sample is loaded onto the column reducing the column lifetime

Evaluation of Loading with Various Organic Solvents



Propyl Gallate

- Dissolved in Water at 1.25 mg/mL
- Dissolved in Organic Solvents at 100 mg/mL

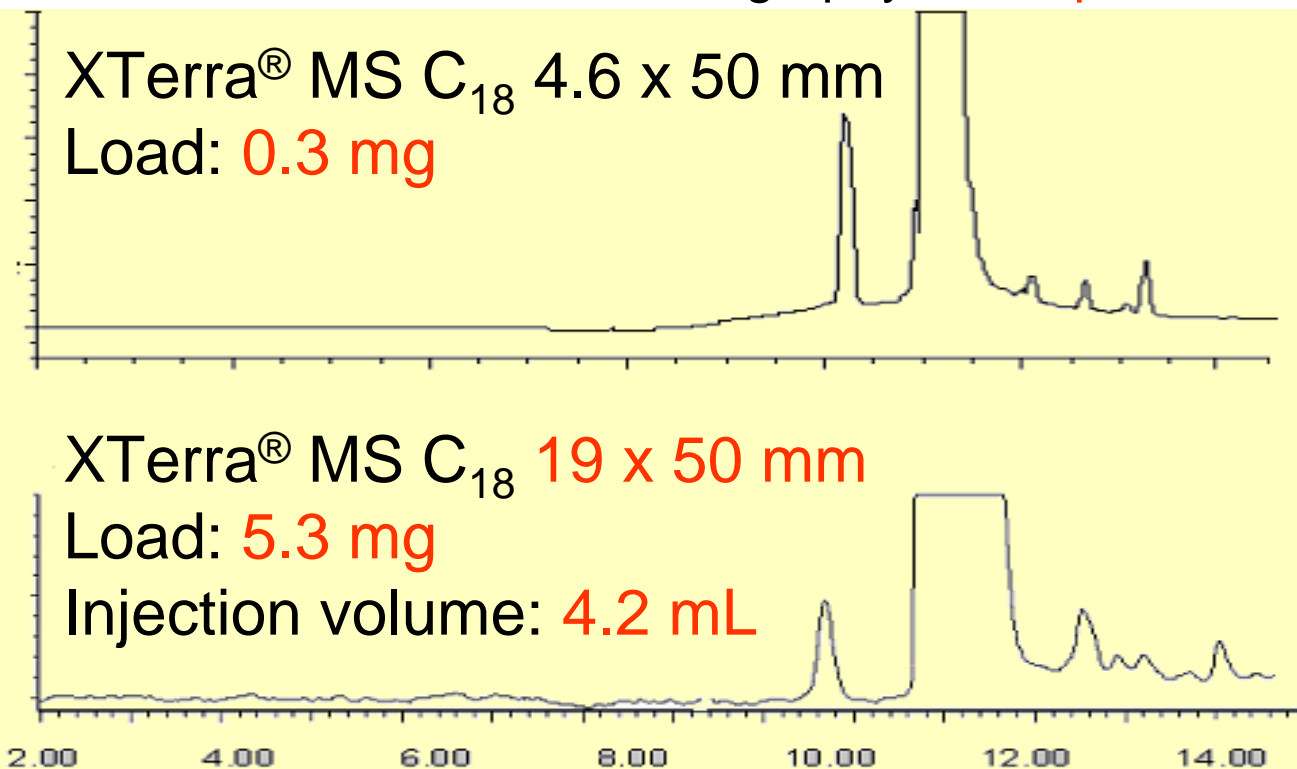
■ Column and conditions:

- XTerra[®] MS C₁₈
- Buffer A: 90/10 DI Water/100 mM Ammonium Formate pH 3.8
- Buffer B: 90/10 Methanol/100 mM Ammonium Formate pH 3.8
- Gradient: 95/5 to 5/95 A/B in 30 column volumes
- UV monitored at 254 nm

Impurity Profile with Sample Dissolved in Water

Propyl Gallate

Sample dissolved in **water**
Chromatography run at **pH 3.8**



A large injection is needed to achieve 5.3 mg loading at preparative scale

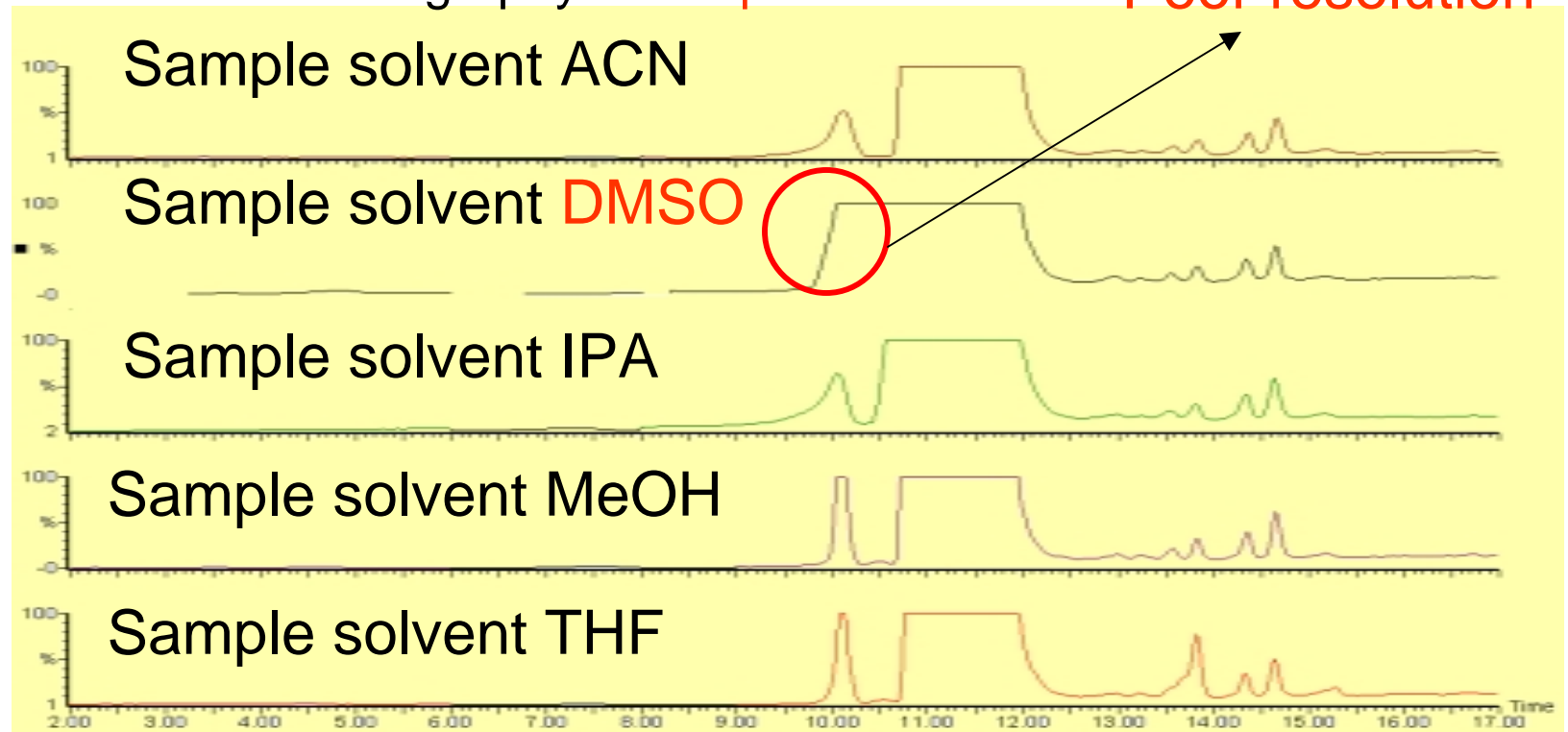
Alternative Sample Solvents are Employed

- To increase solubility of sample, increase loading and decrease injection size, samples are dissolved in organic solvents
- However, the contributions of these solvents can play a significant role in the final chromatography

Sample Solvent Affects Resolution and Peak Shape

Chromatography run at pH 3.8

Poor resolution



XTerra® MS C₁₈ 19 x 50 mm

Loading: 30 mg

Injection volume: 0.3 mL

What causes this phenomena?
Sample solvent strength
or viscous fingering?

Sample Solvent Strength

- Sample solvent strength was evaluated under the following experimental conditions:
 - XTerra[®] MS C₁₈ 4.6 X 50 mm
 - A: DIWater and B: Organic solvent
 - Gradient: 0-100% B in 30 column volumes
 - Flow rate: 1.8 mL/min, UV monitored at 254 nm

| Solvent B | propranolol retention time (min) | toluene retention time (min) | order of elution(*) |
|--------------------|----------------------------------|------------------------------|---------------------|
| Acetonitrile | 2.25 | 5.41 | 3 |
| Dimethyl sulfoxide | 2.50 | 9.76 | 5 |
| Isopropyl alcohol | 1.79 | 5.09 | 1 |
| Methanol | 2.43 | 6.51 | 4 |
| Tetrahydrofurane | 2.21 | 5.41 | 2 |

(*) scale 1-least retained, 5-most retained

DMSO is the weakest solvent, indicating that the lack of resolution is not determined by sample solvent strength

Pure Sample Solvent Viscosity

| Solvent | Viscosity (cP)(*) |
|---------------------------|-------------------|
| Acetonitrile (ACN) | 0.37 |
| Dimethyl sulfoxide (DMSO) | 2.20 |
| Isopropyl alcohol (IPA) | 2.50 |
| Methanol (MeOH) | 0.60 |
| Tetrahydrofurane (THF) | 0.46 |

(*) Neue, Uwe,
"HPLC Columns:
Theory,
Technology and
Practice", Wiley-
VCH, 2nd Ed.,
1997, p.31

If viscous fingering effects decrease resolution due to the viscosity of the pure sample solvent, then a compromised separation should result as the sample solvent viscosity increases. However, while the IPA results are acceptable, that is not the case with DMSO. Therefore, **loss of resolution due to the viscosity of the sample solvent itself is not the case.**

Viscosity Mixture of Sample Solvent and Eluent

- Pressures were recorded when running the previous experiments and the maximum pressure results are shown below.
- Pressure is directly proportional to viscosity

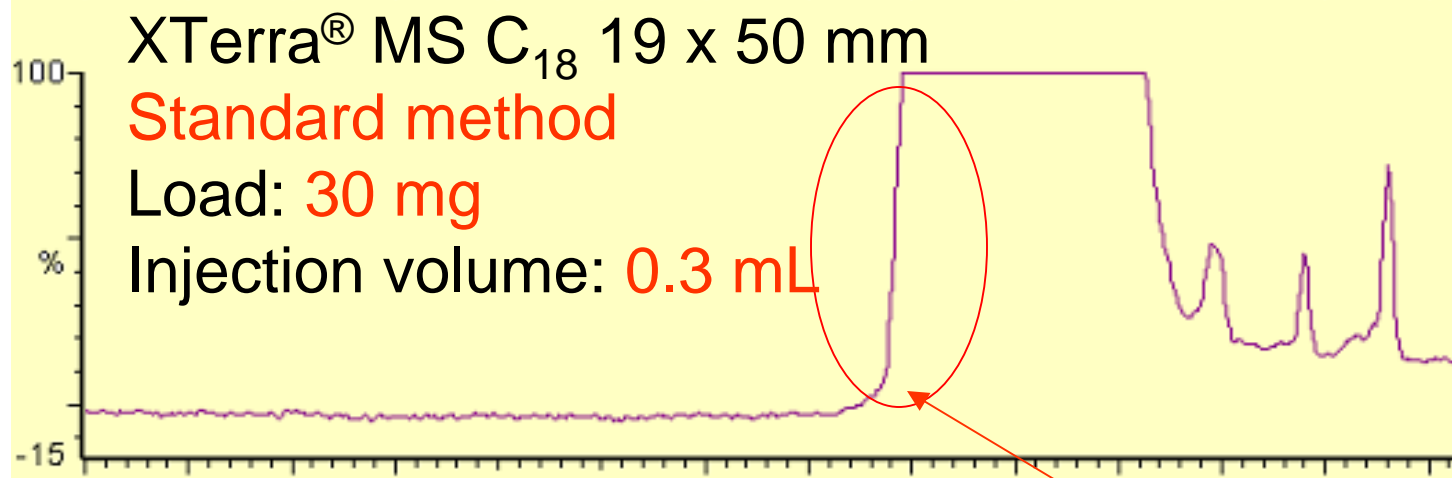
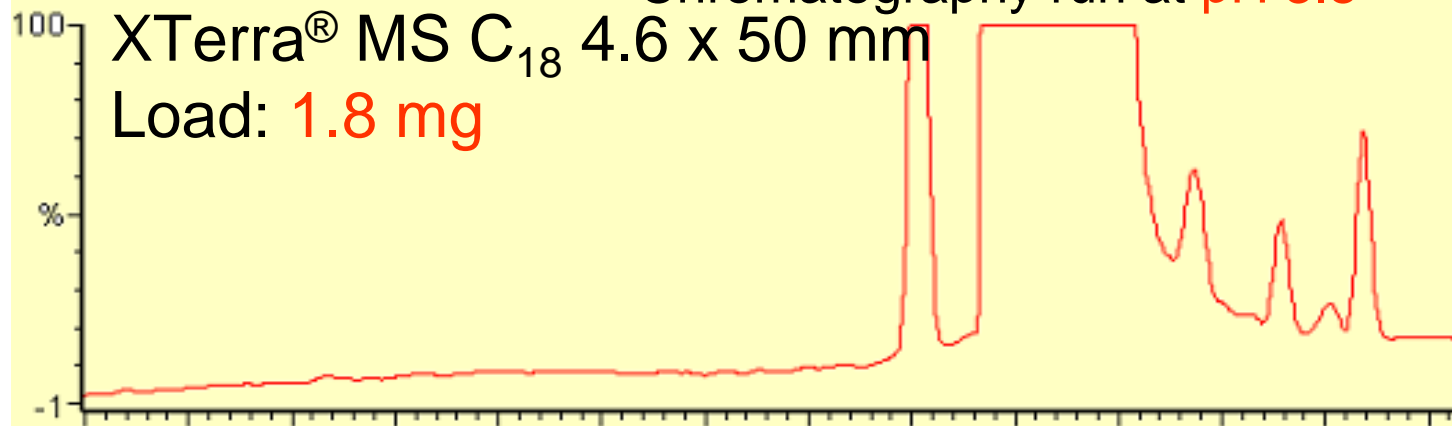
| Solvent | Highest pressure drop across the column(psi) | viscosity ranking |
|--------------------|--|-------------------|
| Acetonitrile | 1120 | 2 |
| Dimethyl sulfoxide | 4400 | 5 |
| Isopropyl alcohol | 3650 | 4 |
| Methanol | 1730 | 3 |
| Tetrahydrofuran | 1105 | 1 |

(*) scale 1-lowest viscosity, 5-highest viscosity

- While IPA has the highest viscosity of the organic solvents tested, the non-idealities of the mixture DMSO/water cause the highest viscosity in the experiments.

Impurity Profile at Low pH

Propyl Gallate Sample dissolved in **DMSO**
Chromatography run at **pH 3.8**



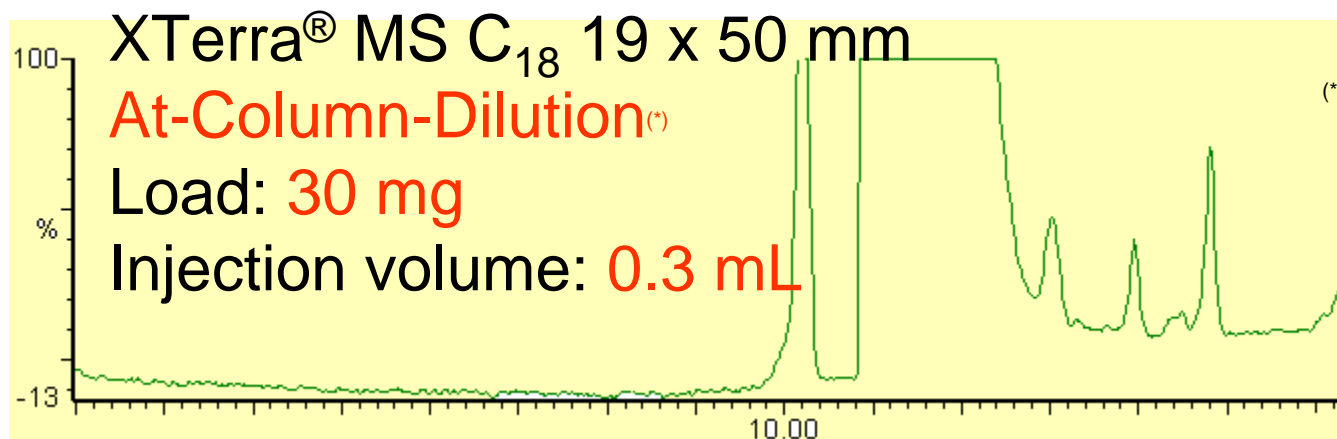
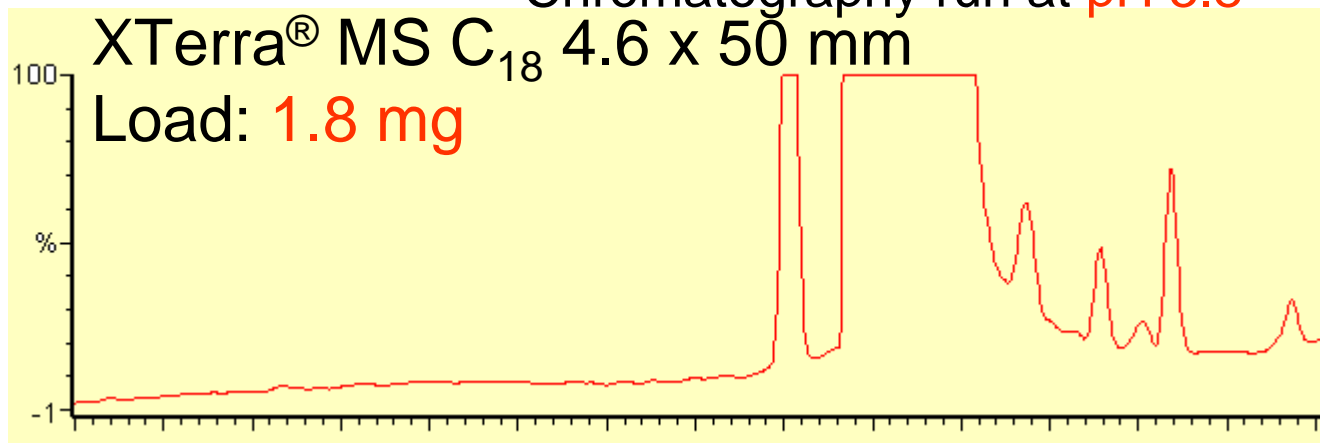
Poor resolution

Evaluating the Chromatographic Results

- The non-ideal mixture of DMSO and initial water rich mobile phases generate such high viscosities in the preparative column that a “viscous fingering” type of effect is created resulting in poor resolution of the chromatographic peaks.
- An alternative method of injecting samples onto the column is urgently needed as a significant percentage of drug candidates that need to be isolated and purified are dissolved in DMSO.

At-Column-Dilution Improves Impurity Isolation

Propyl Gallate Sample dissolved in **DMSO**
Chromatography run at **pH 3.8**



(*) Patent Pending

5.6X increase in mass and **93%** injection volume reduction by using DMSO as sample solvent and the at-column-dilution method

Conclusions

- The use of at-column-dilution when loading basic compounds at high pH as the HCl salt results in a substantial improvement of chromatographic separations
- At-column-dilution is the preferred method when purifying samples dissolved in DMSO
- The at-column dilution method results in enhanced loadability for ionizable compounds when they are loaded onto the column in a non-ionized form