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Increasing Production Rate of Basic Compounds by Performing Preparative Chromatography at High pH

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Abstract

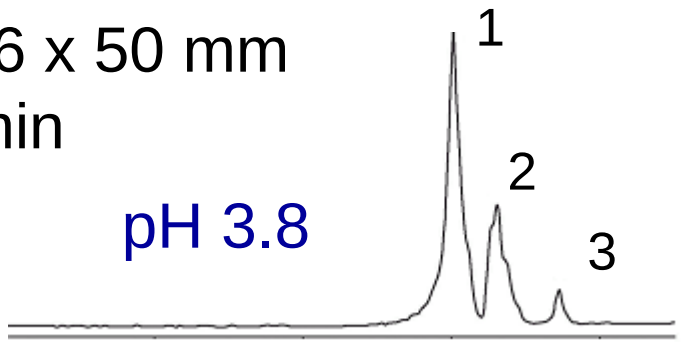
Reversed-phase chromatography of basic compounds has traditionally been performed at acidic pH to minimize secondary interactions and improve peak shape. In the last decade, the advent of higher purity silica and improved bonding technology has permitted the use of more neutral pH for basic compounds. The latest advance has been the development of hybrid particle packings that allow chromatography at high pH with good column lifetime. We have recently found that running preparative chromatography of basic compounds at high pH leads to substantial gains in loadability compared to running at neutral or acidic pH. In particular, converting the solutes to neutral species permits at least 50 times higher loadability, such that 500 mg can be loaded on a 19 X 50 mm column. The implications of these results are addressed.

Loading of Bases at Low pH

Diphenhydramine (1)
Oxybutynin (2)
Terfenadine (3)

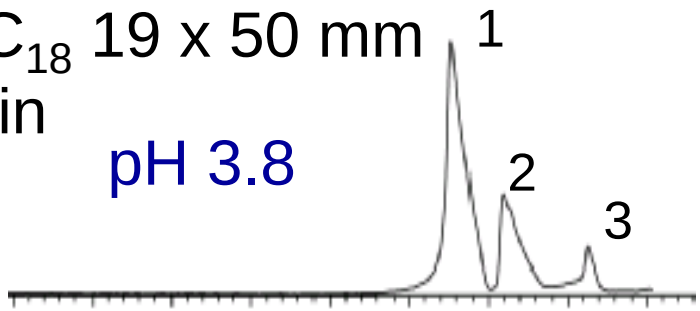
XTerra® MS C_{18} 4.6 x 50 mm
Flow rate 1.8 mL/min
Load 0.4 mg

pH 3.8



XTerra® Prep MS C_{18} 19 x 50 mm
Flow rate 31 mL/min
Load 6 mg

pH 3.8



Gradient: A: 90/10 Water/100 mM Ammonium Formate pH 3.8

B: 80/10/10 ACN/Water/ 100 mM Ammonium Formate pH 3.8

Gradient slope: 95/5 A/B to 5/95 A/B in 10 minutes; UV: 254 nm

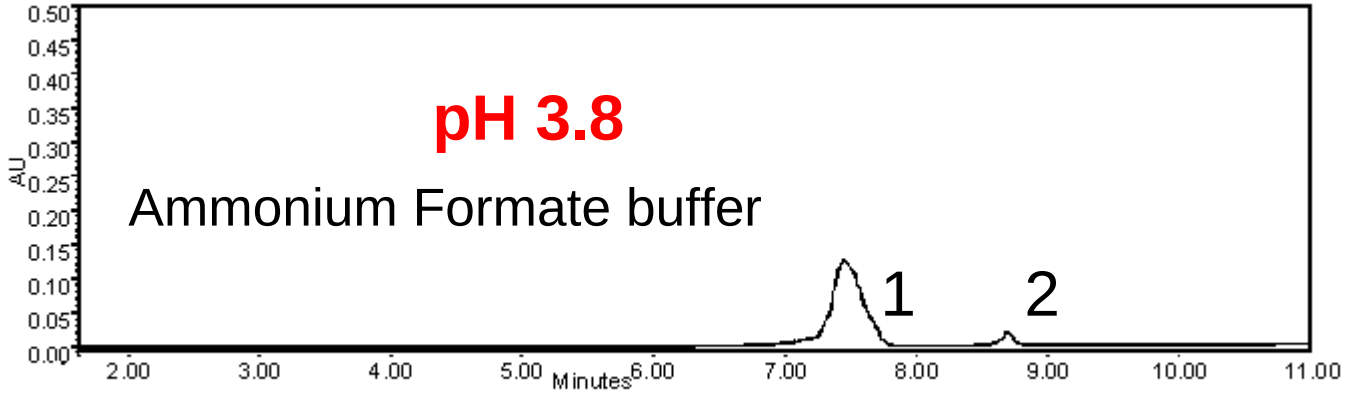
Peak Shape and Retention Comparison

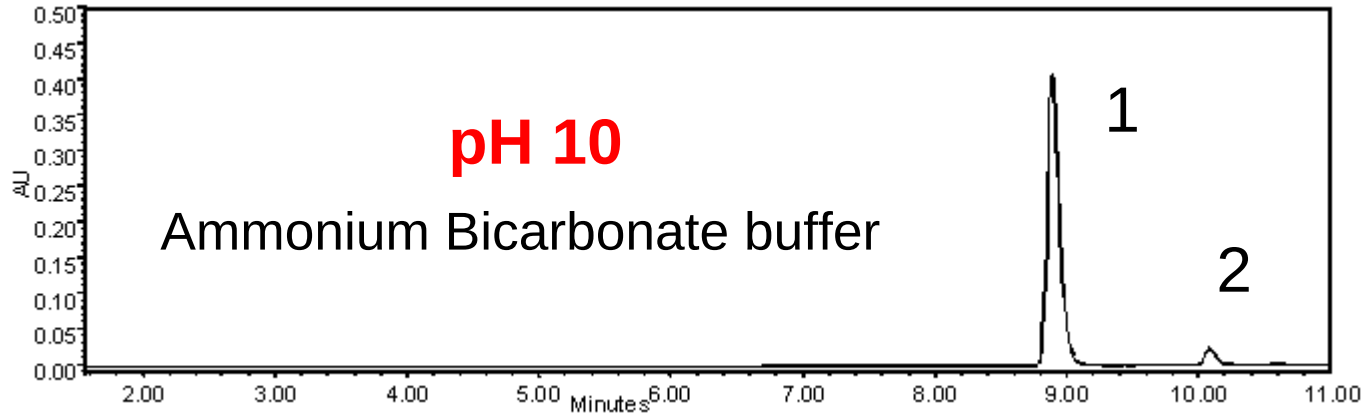
- It is logical to assume that once we have selected our column for selectivity, if we could improve peak shape we could also improve loading capacity

- How can we improve the peak shape for our basic analytes example?

Peak Shape and Retention Comparison: Basic Compounds at Low and High pH

XTerra® Prep MS C_{18} 19 x 50 mm, 5 µm

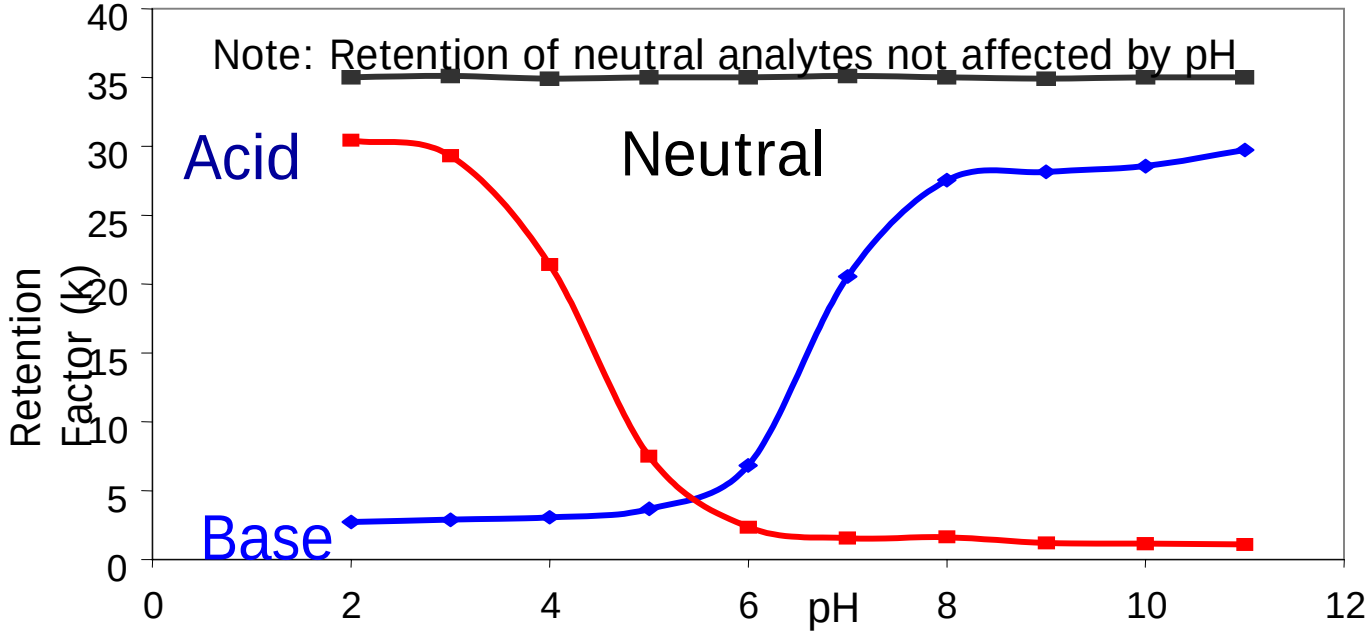




Analytes: 1. Diphenhydramine (2.5 mg/mL) 3 mg Load

2. Terfenadine (0.15 mg/mL) 0.18 mg Load

Retention Map Theory



The increase in loadability shown is a generic phenomena that has been proven employing XTerra® where the loading difference between the ionized and non-ionized form of the compound varies by 50 fold.(*)

(*) U.D. Neue *et al*., American Laboratory, November 1999, 31 (22), p. 36-39

Peak Shape and Retention Comparison: Basic Compounds at Low and High pH

- For basic compounds:
  - The peak shape improves at high pH
  - The retention increases at high pH
- This implies that, due to improved peak shape it is possible to load more material onto the column under high pH conditions
- How much more ???

Loading of Bases

XTerra® Prep MS C_{18}, 19 x 50 mm

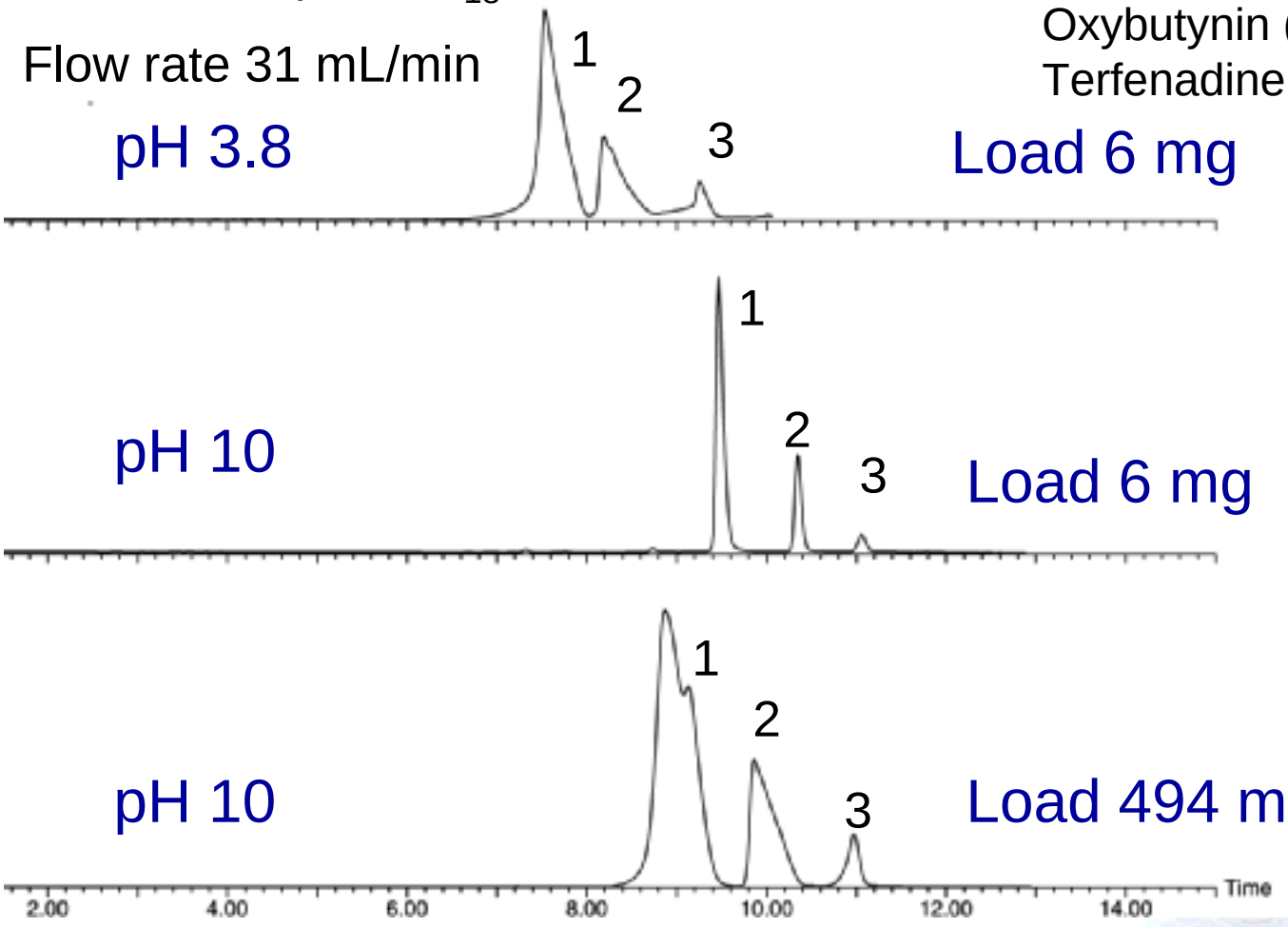
Flow rate 31 mL/min

Diphenhydramine (1)
Oxybutynin (2)
Terfenadine (3)

pH 3.8 — Load 6 mg

pH 10 — Load 6 mg

pH 10 — Load 494 mg



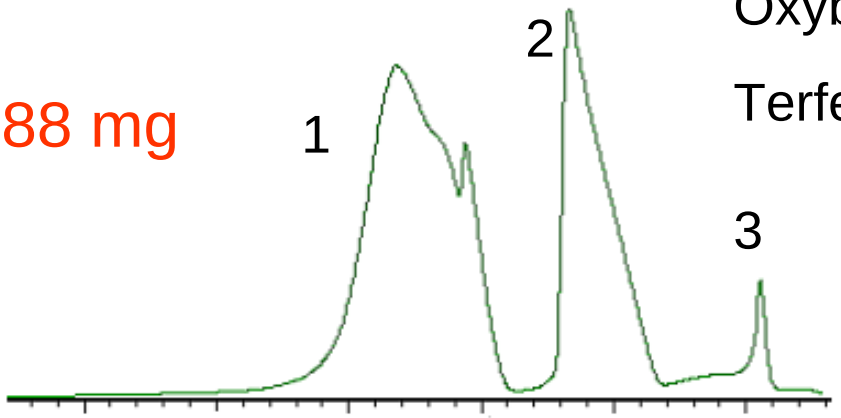
Increased at least 60X loading in the same column!

Scalability of Bases at High pH

Double column size, double load

XTerra® Prep MS C_{18}, 19 x 100 mm
Flow rate 31 mL/min

Load 988 mg



Diphenhydramine (1)

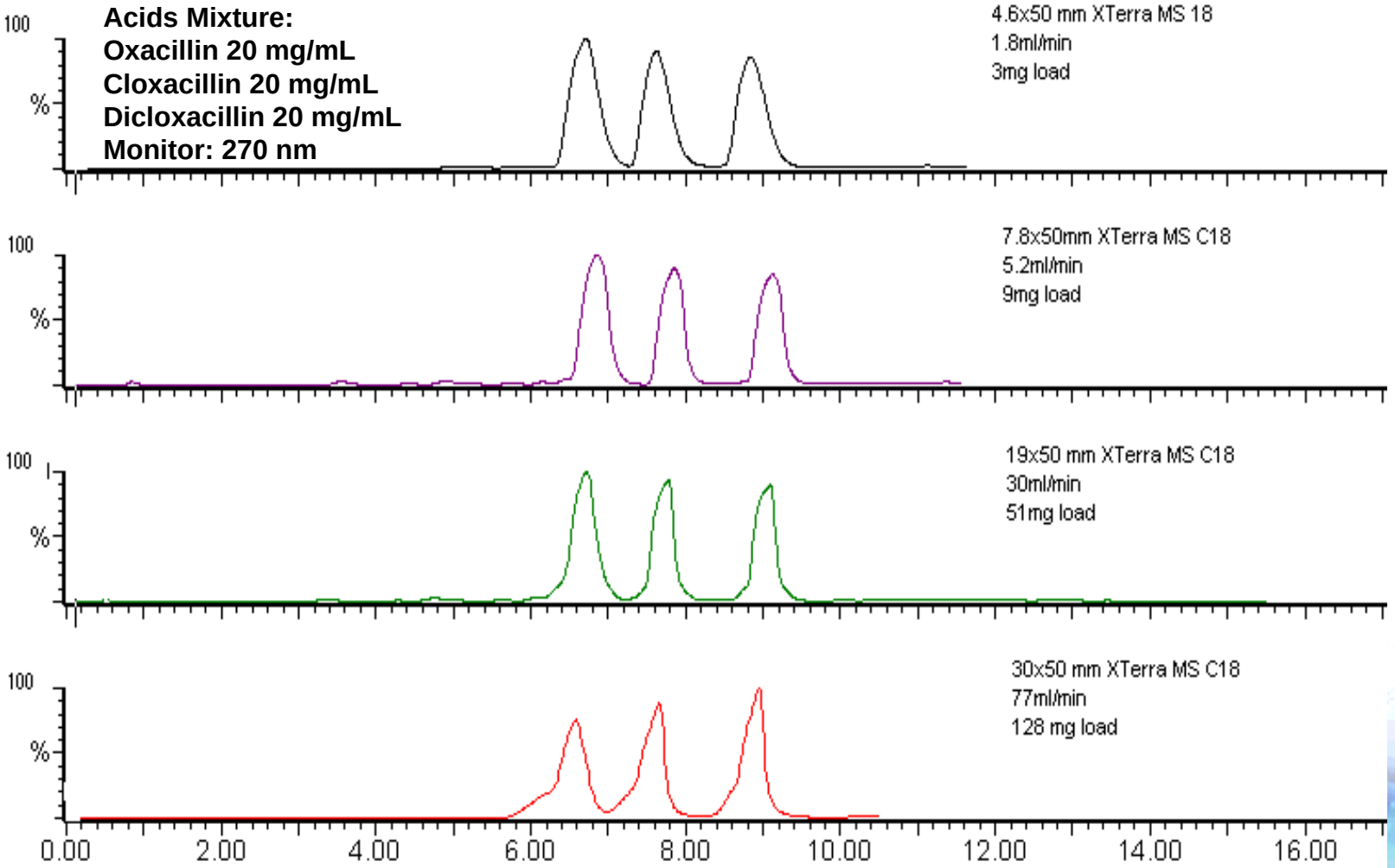
Oxybutynin (2)

Terfenadine (3)

Scalability of Acids at Low pH

Buffer A: 10 mM Ammonium Formate pH 3.8 in H_2O
Buffer B; 10 mM Ammonium Formate pH 3.8 in 95:5 ACN:H_2O
Gradient from 90:10 to 40:60 A:B in 15 minutes

Acids Mixture:
Oxacillin 20 mg/mL
Cloxacillin 20 mg/mL
Dicloxacillin 20 mg/mL
Monitor: 270 nm

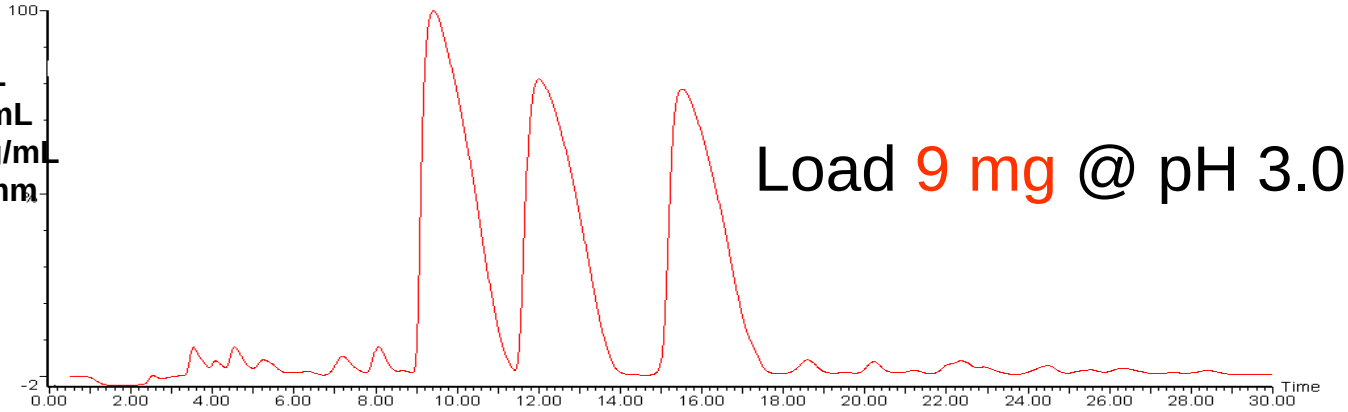


Scalability of Acids at Even Lower pH: Increase in Loadability

4.6 x 50 mm
Load 3 mg @ pH 3.8

Buffer A: 10 mM Ammonium Formate pH 3.0 in H_2O
Buffer B; 10 mM Ammonium Formate pH 3.0 in 95:5 ACN:H_2O
Gradient from 70:30 to 56:44 A:B in 18 minutes

Acids Mixture:
Oxacillin 20 mg/mL
Cloxacillin 20 mg/mL
Dicloxacillin 20 mg/mL
Column: 4.6 X 50 mm
Monitor: 270 nm



Load 9 mg @ pH 3.0

Loading increased 3X by lowering the buffer pH from 3.8 to 3.0
It is possible to load 384 mg on a 30 x 50 mm column

Increase in Loadability when Compound Loaded in Non-Ionic Form

- Ionized sample compound 1X Load
- Non-ionic sample compound > 20X Load
- Examples:
  - Doxylamine
  - Diphenhydramine
  - Oxybutinin
  - Terfenadine
  - Propyl Gallate
  - Oxacillin
  - Cloxacillin
  - Dicloxacillin

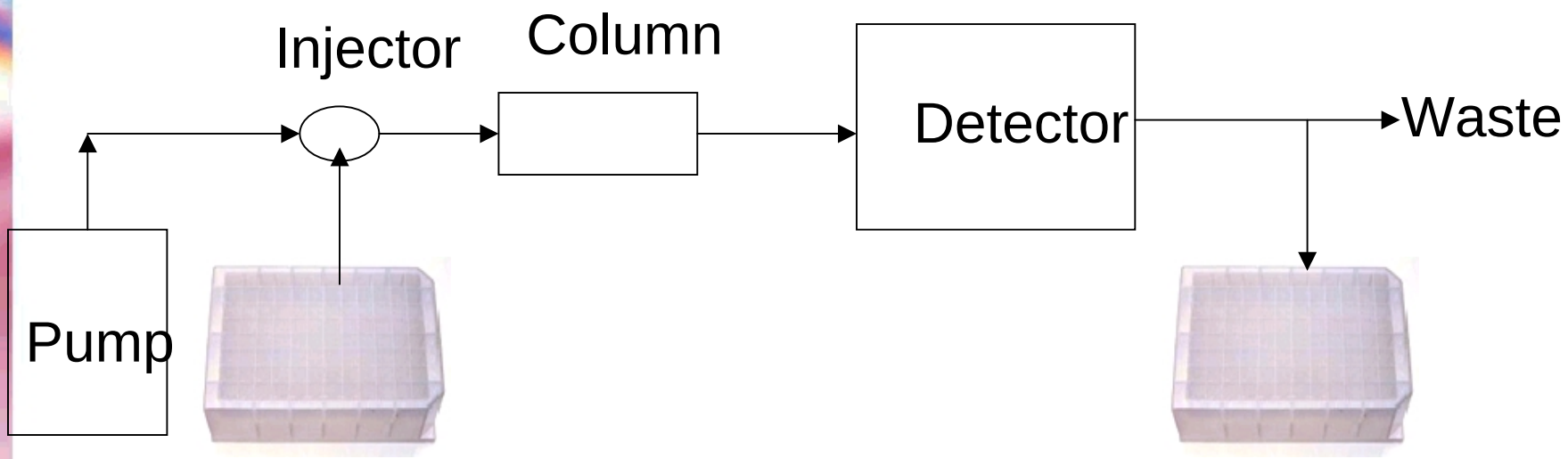
What Size Column is Really Necessary?

- While it has been shown that up to 500 mg can be loaded onto a 19 x 50 mm column, it is not always necessary to load that much.
- As loadability increases significantly when carrying out chromatography with ionizable compounds in their neutral state, then it is possible to consider reducing the column size.

Scaling to Smaller Columns Allows:

- Faster chromatography while maintaining resolution and peak purity
- Peak volume reduction leading to reduced post-purification sample handling time including dry-down of fraction
- Less expensive column
- Depending on the application, how far is it possible to downsize?
- Plate-to-plate mapping - injecting from a 96-well plate and collecting fractions in another 96-well plate - could it now be possible?

Plate-to-Plate Mapping



Samples to be purified

2 mL 96 well plate

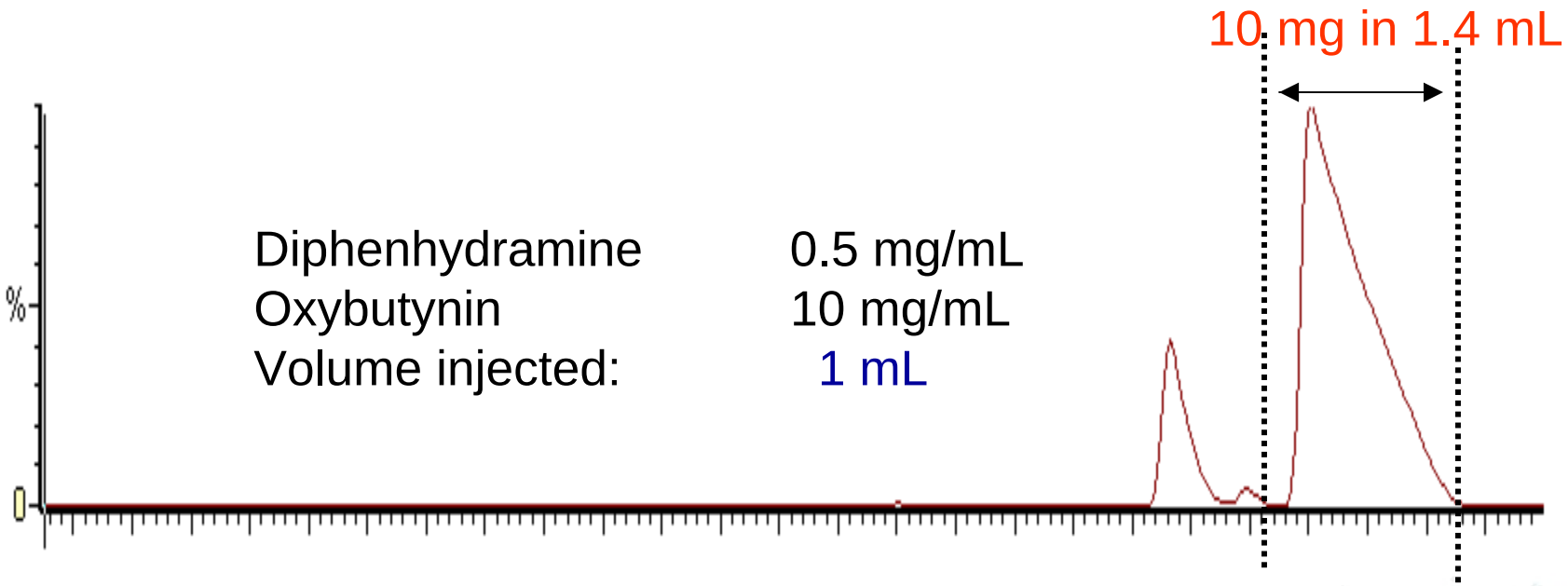
Fractions collected

2 mL 96-well plate

If sample loaded is on the order of 10 mg,
Analytical columns can be employed

Pure Material in One Fraction

XTerra® MS C_{18} 4.6 x 50 mm



This volume can be collected in one fraction in one 2 mL well

Conclusions

- We have shown that loadability increases dramatically when carrying out chromatography with ionizable compounds in their neutral state
- As loadability increases, it is possible to purify compounds in less runs and use smaller columns, decreasing fixed and operational costs significantly
- Plate-to-plate mapping for loadings within the 10 mg range is now possible using analytical sized columns and equipment, decreasing costs as well as fraction handling