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Effect of Peak Symmetry on Analytical Figures of Merit Used in Pharmaceutical Assay Validation

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Abstract

Pharmaceutical scientists are required to verify the suitability of United States Pharmacopeia methods under actual conditions of use. This validation process establishes that the performance of the method meets the requirements for the intended analytical applications. Performance characteristics are expressed in terms of analytical parameters which include limits of detection and limits of quantitation. For HPLC methods the limits of detection and quantitation are affected by peak symmetry. A higher signal-to-noise ratio is obtained for symmetrical than asymmetrical peaks. The more peak tailing the lower the peak height for a given sample load which results in a lower signal-to-noise ratio. The data in this paper show that reversed-phase columns which give symmetrical peaks for all analytes including basic pharmaceuticals allow higher signal to noise ratios to be achieved. For example, the Symmetry® C_8 column gave symmetrical peaks for three procainamides (USP tailing factor ≤1.4) allowing a signal to noise ratio of 18 to be achieved at 0.5 ng of sample on column. Another new generation column which gave tailing factors of ≥2.4 for these compounds had a signal-to-noise ratio of only 4 at the same concentration. Data will be presented to show that more symmetrical peaks give lower limits of detection and quantitation for drugs and their impurities or degradation products.

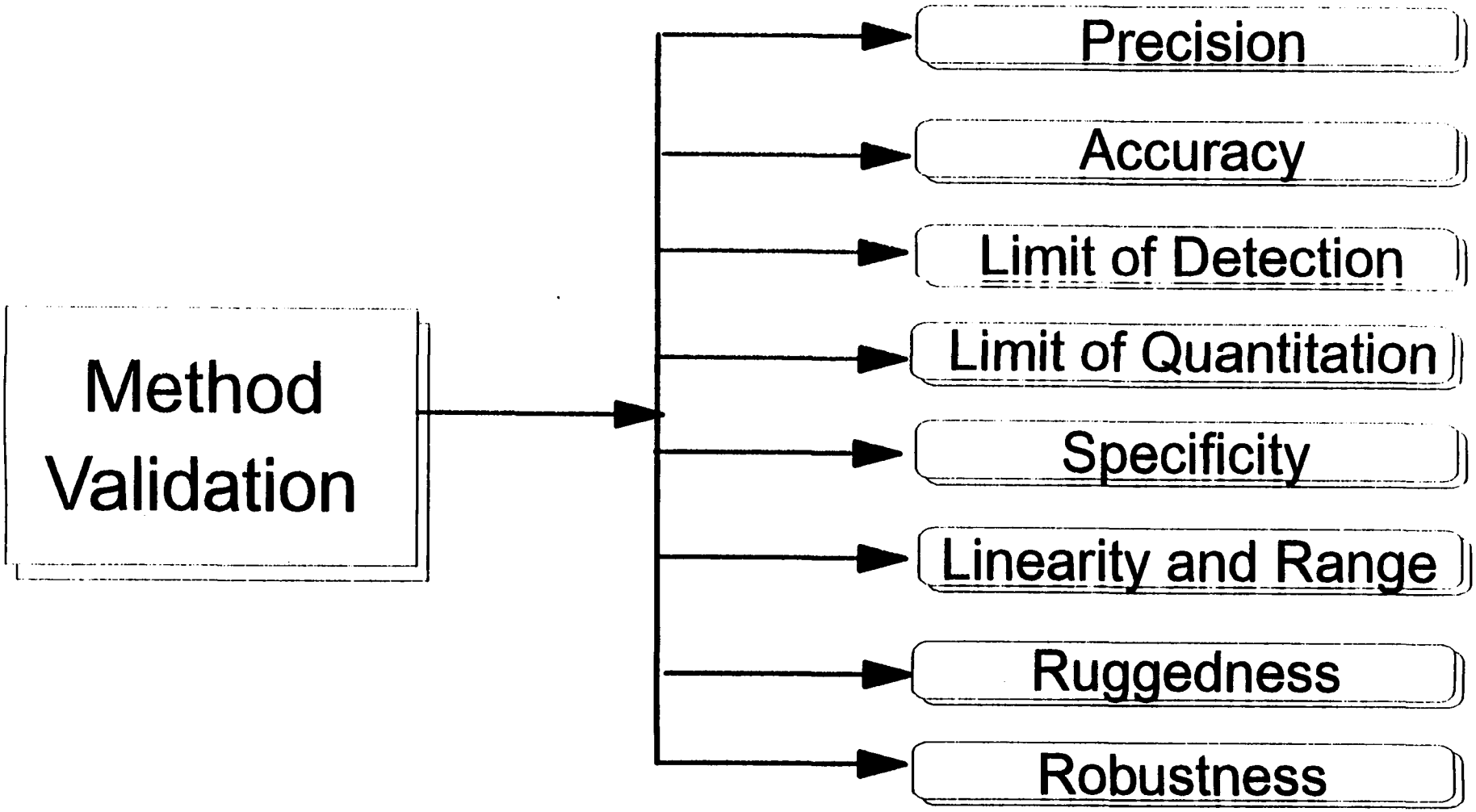
Introduction

The objective of validation of an assay is to demonstrate by laboratory studies that it meets the requirements for the intended analytical application.

The typical analytical criteria used in assay validation are given in the United States Pharmacopeia, and in the guidelines from the Food and Drug Administration and the International Conference on Harmonization (European).

Different test methods require different validation schemes. The analytical methods for bulk drugs and preservatives require all the criteria listed except limits of detection and quantitation. Assays for impurities and degradants require all the criteria listed. This paper focuses on the limits of detection and quantitation as impacted by peak symmetry and column dimensions.

Method Validation Criteria



Column Requirements

- High batch-to-batch reproducibility
- High column-to-column reproducibility
- Symmetrical peaks
- High efficiency
- Broad choice of column dimension

Introduction (cont.)

Column quality impacts the ability to validate the assay. Therefore to guarantee consistent results, what one would like to see in a column is good column-to-column and batch-to-batch reproducibility. Symmetrical peaks and high column efficiencies are important. The ability to obtain good peak shapes for different compounds, including bases, results in more sensitive and more rugged assays.

The Symmetry columns are designed to make the method validation process easier and to give more robust and rugged assays.

The Symmetry packings were developed to meet the needs of the pharmaceutical scientist in three areas: peak symmetry independent of mobile phase pH and nature of drug, reproducibility and column lifetime and efficiency.

Symmetry™

- Standard-Setting Process Controls and Specifications
- High Purity Reagents in Silica Synthesis and Surface Modification
- Improved Bonding Technology
- Improved Packing Technology

Resulting in

- Unmatched Batch-to-Batch Reproducibility
- Excellent Peak Symmetry Independent of pH
- Excellent Column Lifetime and Efficiency

Limits of Quantitation and Detection

- LOD
  - the lowest concentration of an analyte in a sample that can be detected under the experimental conditions.
    - » S/N ratio of 2:1 or 3:1

- LOQ
  - the lowest concentration of the analyte that can be determined with acceptable precision and accuracy under the experimental conditions.
    - » S/N ratio generally 10:1

LOQ and LOD Relationship to Column Performances

- The LOD and LOQ are proportional to the concentration at peak maximum, C_{max}.
- The equation shows the parameters that affect the concentration at peak maximum.
- Retention factor, k, is seldom changed since a change in this parameter potentially affects selectivity.
- The fact that asymmetry affects sensitivity is often overlooked. If the tailing is measured at 10% of peak height, β is 4; a peak with a tailing factor of 1.5 results in 3X lower sensitivity.

LOQ and LOD Relationship to Column Performances

- LOQ and LOD can be expressed by:

$$C_{max} = \frac{4}{\varepsilon\pi\sqrt{2\pi}} \frac{1}{\left[1 + \beta\ (T_a - 1)\right]} \frac{\sqrt{N}}{L\,d_c^2} \frac{m_D}{(1 + k)}$$

Peak Shape

Column Dimension and Performance

Retention

Enhancing Sensitivity

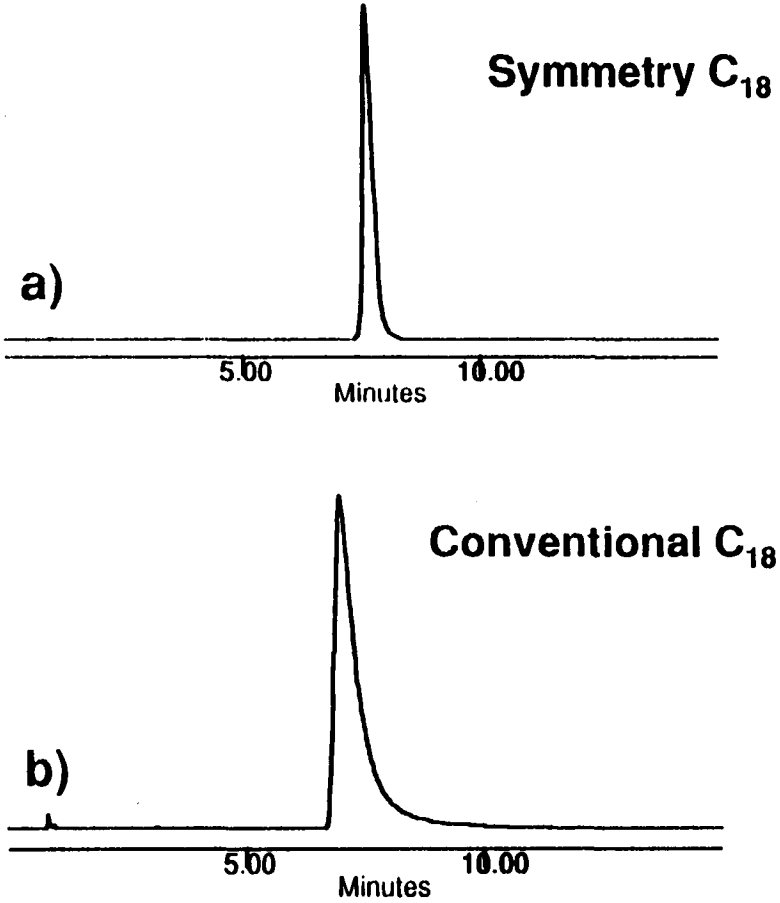
Sensitivity can be enhanced by:

- **increasing efficiency**
- **decreasing asymmetry**
- **reducing column diameter**
- **using shorter columns**
- **decreasing detector noise**
- **using more sensitive detection modes**
- **decreasing capacity factor**

Effect of Peak Symmetry on LOD and LOQ

- Several aspects of the analysis of tamoxifen demonstrate the impact of peak symmetry on sensitivity.
- Tamoxifen exhibits a good peak shape on Symmetry columns. A conventional base-deactivated column exhibits excessive tailing for tamoxifen even at pH 3.
- Using the parent compound as a model, the amount close to the limits of quantitation was determined. At 0.25 μg/ml there is a marked difference in the signal/noise ratio between the Symmetry column and the conventional column.
- The third tamoxifen slide shows that quantitation of impurities at 0.1% of the parent drug is impacted by peak shape. Peak asymmetry has a negative effect on the ability to perform the analysis.

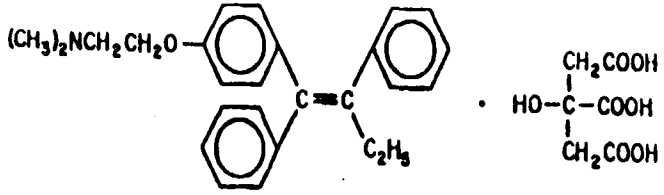
Tamoxifen: Influence of Asymmetry



	USP Plates	Tailing Factors
a.)	6500	1.4
b)	1080	3.0

Conditions:

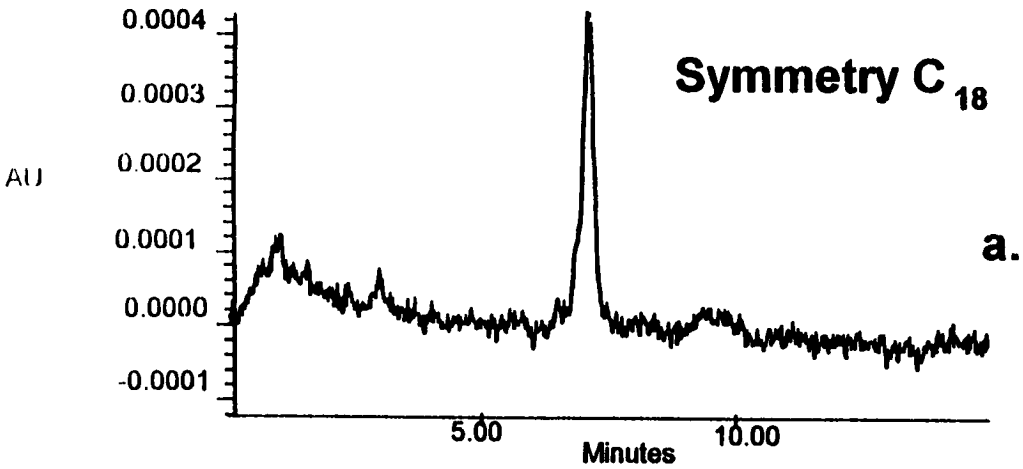
Columns:	a) Symmetry C_{18} 3.9 mm x 150 mm b) Zorbax Rx C_{18} 4.6 mm x 150 mm
Mobile Phase:	50 mM potassium phosphate, pH 3/ acetonitrile 55:45
Flow Rates:	a)1 ml/min b) 1.4 ml/min
Detector:	240 nm

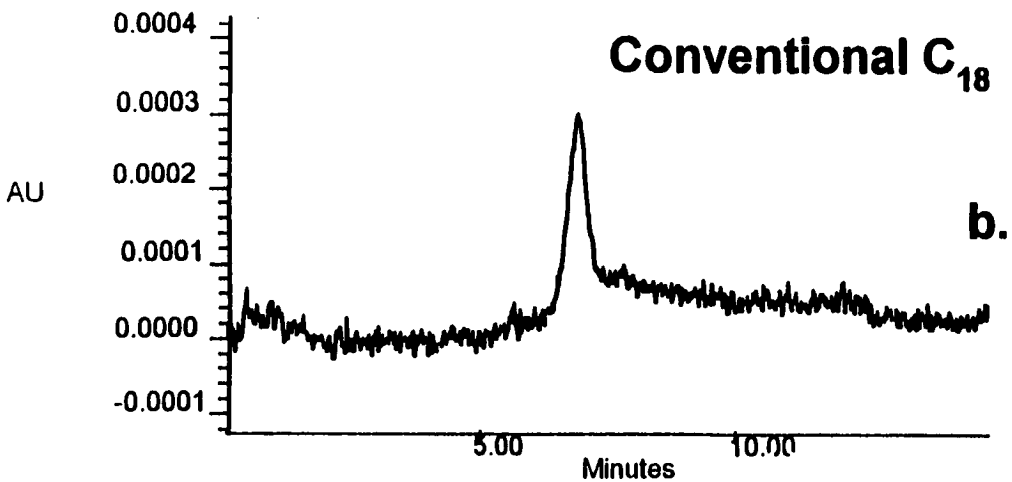


1. Tamoxifen citrate

Tamoxifen: Influence of Asymmetry on Sensitivity

0.25 µg/ml





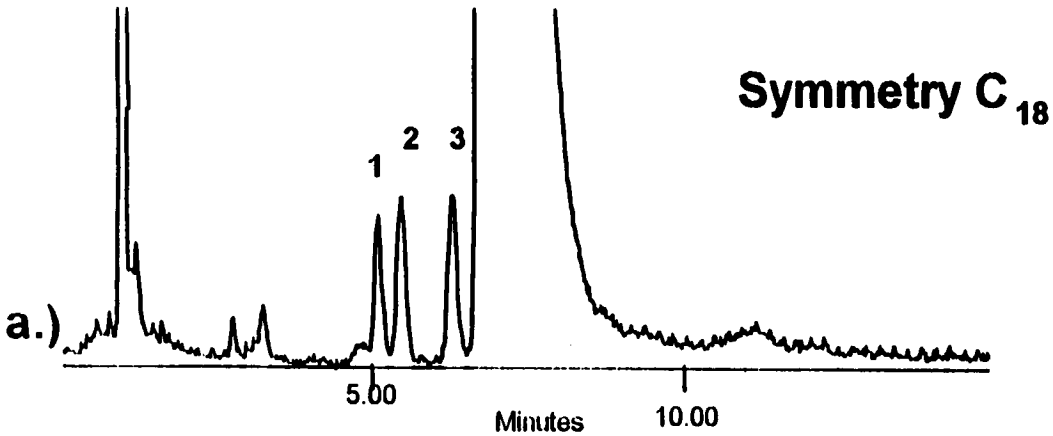
Signal/Noise Ratio

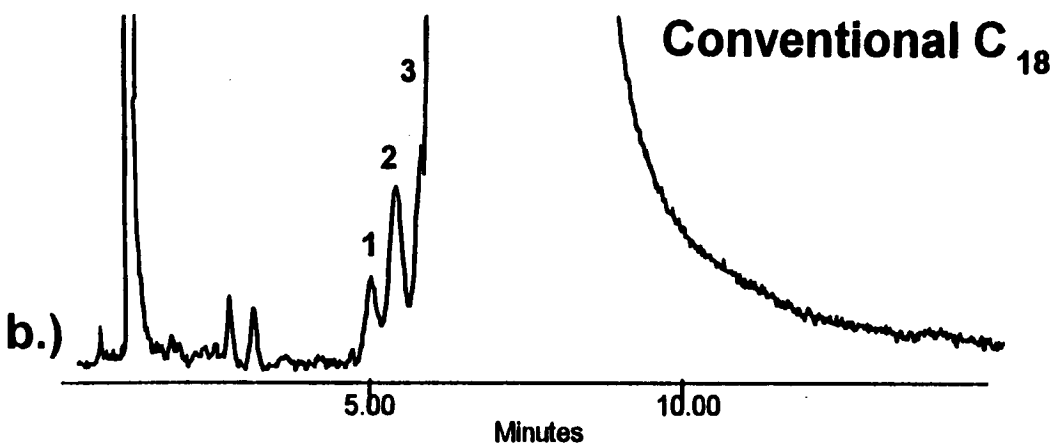
a)	11.0
b)	6.5

Conditions:

Columns:	a) Symmetry C_{18} 3.9 mm x 150 mm b) Zorbax Rx C_{18} 4.6 mm x 150 mm
Mobile Phase:	50 mM potassium phosphate, pH 3/ acetonitrile 55:45
Flow Rates:	a) 1.0 ml/min b) 1.4 ml/min
Detector:	240 nm
Injection Vol.:	a) 10 µl b) 14 µl

Tamoxifen: Influence of Asymmetry on Impurity Profile





	USP Plates	Tailing Factors
a)	6500	1.4
b)	1080	3.0

Conditions:

Columns:	a) Symmetry C_{18} 3.9 mm x 150 mm b) Zorbax Rx C_{18} 4.6 mm x 150 mm
Mobile Phase:	50 mM potassium phosphate, pH 3/ acetonitrile 55:45
Flow Rates:	a) 1.0 ml/min b) 1.4 ml/min
Detector:	240 nm
Sample:	600 µg/ml, 10 µl injection

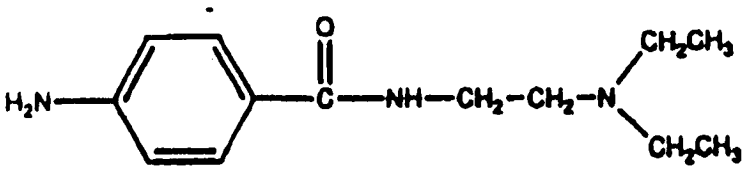
Effect of Peak Symmetry and Efficiency on LOD and LOQ

- Symmetry C_8 column gives excellent peak shapes (USP tailing factors ≤ 1.4) for the basic procainamides with a pH 6 mobile phase. A conventional C_8 column gives poor peak shapes (tailing factor ≥ 2.4) for the three compounds at this pH.

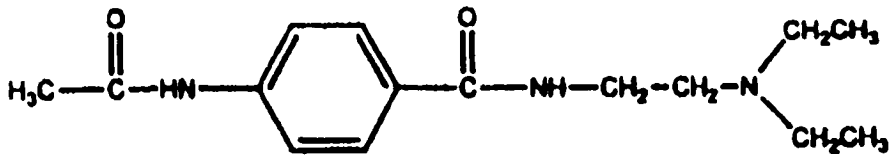
- The more symmetrical peaks result in a higher signal to noise ratio. At low levels (0.1 μg/ml each), similar to those of impurities or degradants, the Symmetry column gives a 4.5 fold higher signal to noise ratio than the conventional column.

Procainamides Conditions and Structures

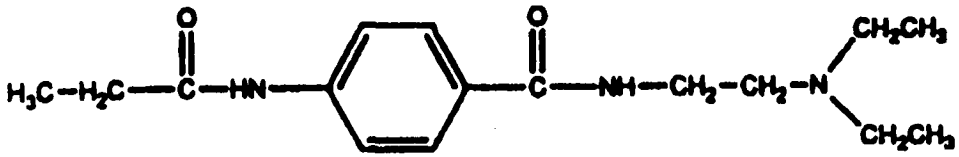
- **Chromatography conditions:**
  - Columns: a. Symmetry C_8 3.9 mm x 150 mm
    b. Zorbax Rx C_8 4.6 mm x 150 mm
  - Mobile Phase: 20 mM potassium phosphate, pH 6.0/ acetonitrile a. 90:10; b. 84:16
  - Flow rate: a. 1mL/min; b. 1.4 mL/min
  - Detection: 254 nm
  - Sample: 0.1 μg/mL of each procainamide, N-acetylprocainamide, and N-propionylprocainamide (in order of elution)



1. procainamide

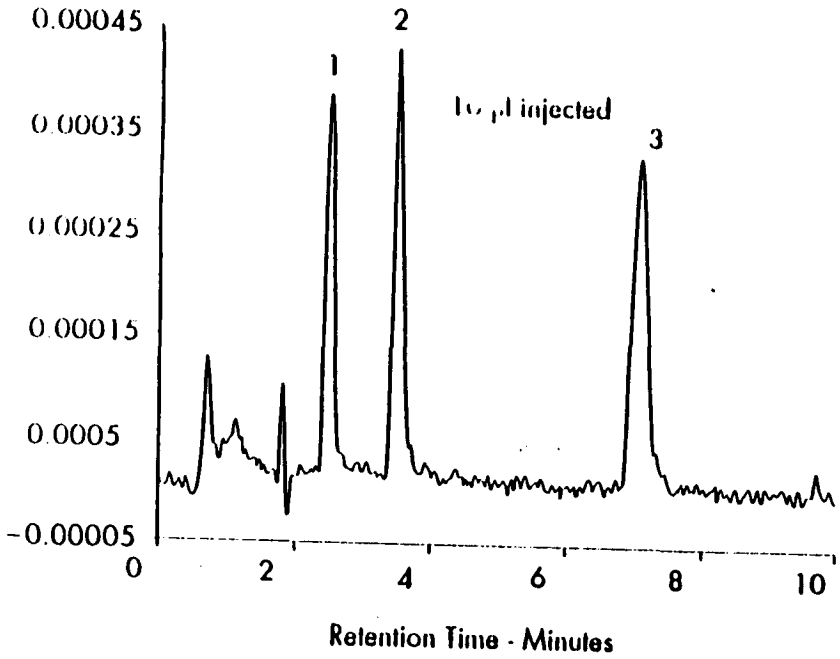


2. N-acetylprocainamide

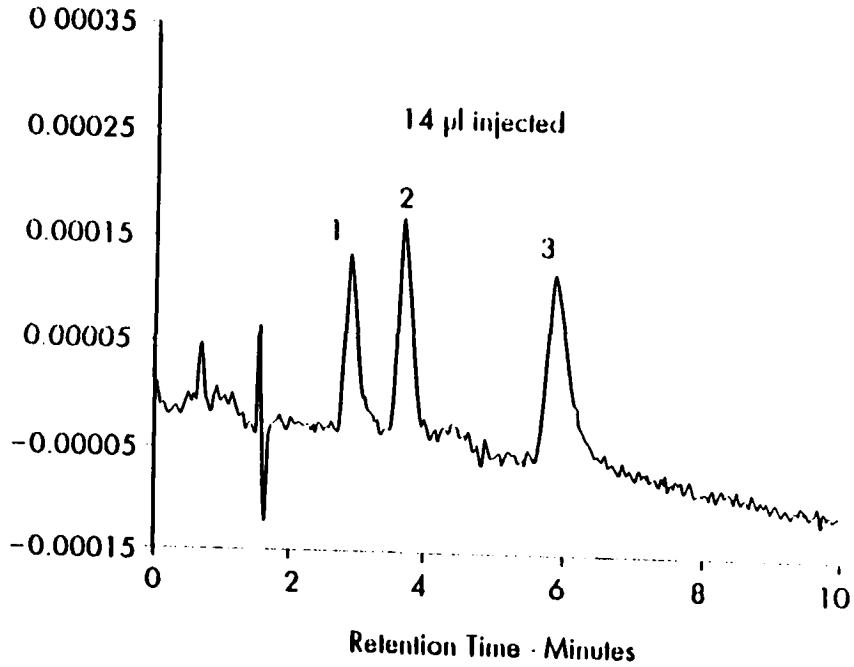


3. N-propionylprocainamide

Symmetry C_8



Zorbax Rx C_8



Trade-off between Sensitivity and Resolution

- Increase in injection volume increases sensitivity
- Increase in injection volume can decrease resolution
- Decrease in column diameter increases sensitivity
- Decrease in column diameter can decrease resolution

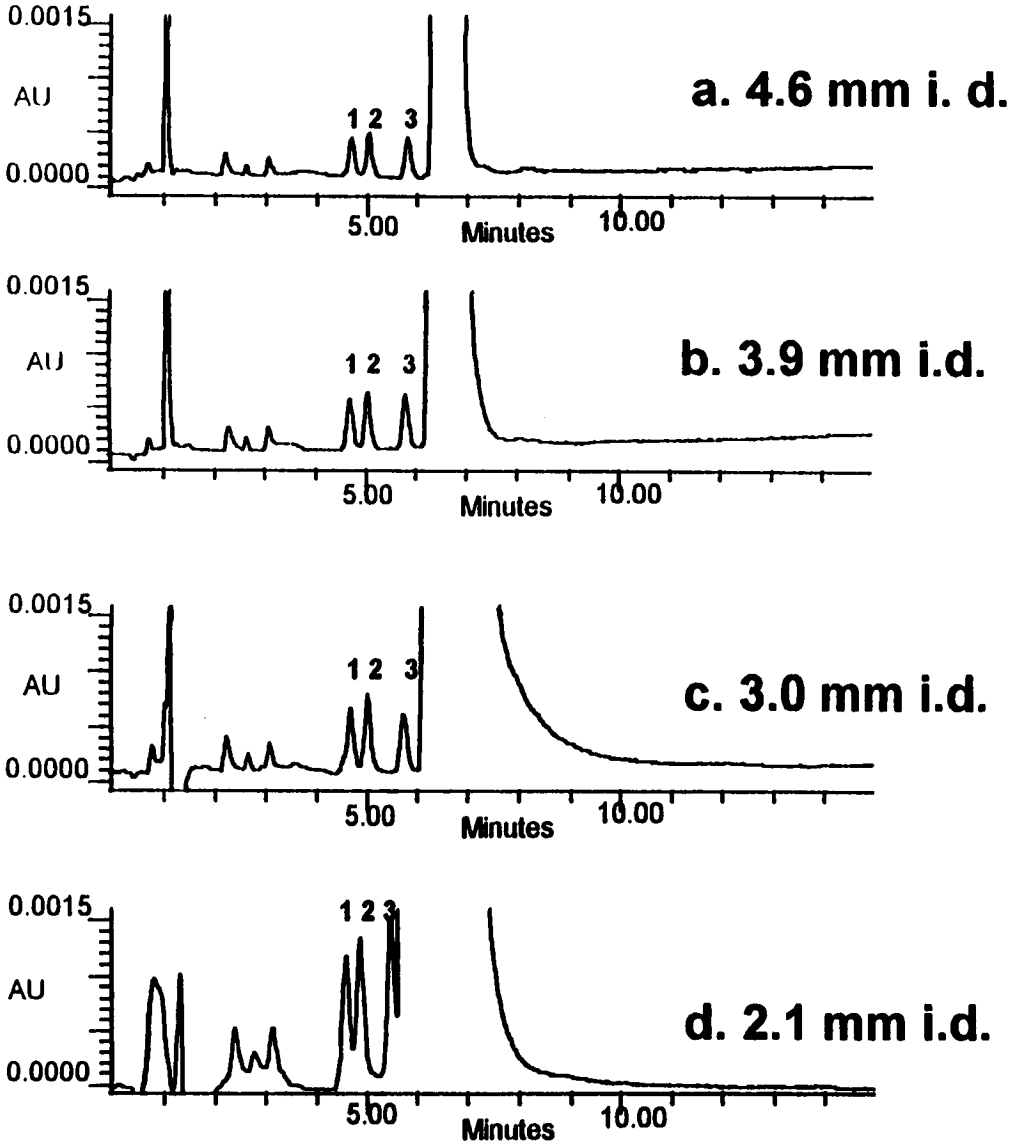
Sensitivity and Resolution as a Function of Column Diameter

- The injection of a constant volume of tamoxifen on different diameter columns shows the trade-off between sensitivity and resolution.

- The peak heights for the tamoxifen impurity profile increases as column diameter decreases for the same mass injected.

- There is a loss in resolution that is especially noticeable on the 2.1 mm i.d. column. The cause of the loss of resolution can be volume and/or mass overload.

Sensitivity as a Function of Column Diameter



Conditions:

Columns:	Symmetry C_{18} 150 mm length
Mobile Phase:	50 mM potassium phosphate, pH 3.0/acetonitrile 55:45
Flow Rates:	a. 1.4 ml/min b. 1.0 ml/min c. 0.60 ml/min d. 0.29 ml/min
Detection:	240 nm
Sample:	tamoxifen, 600 µg/ml, 7 µl injection

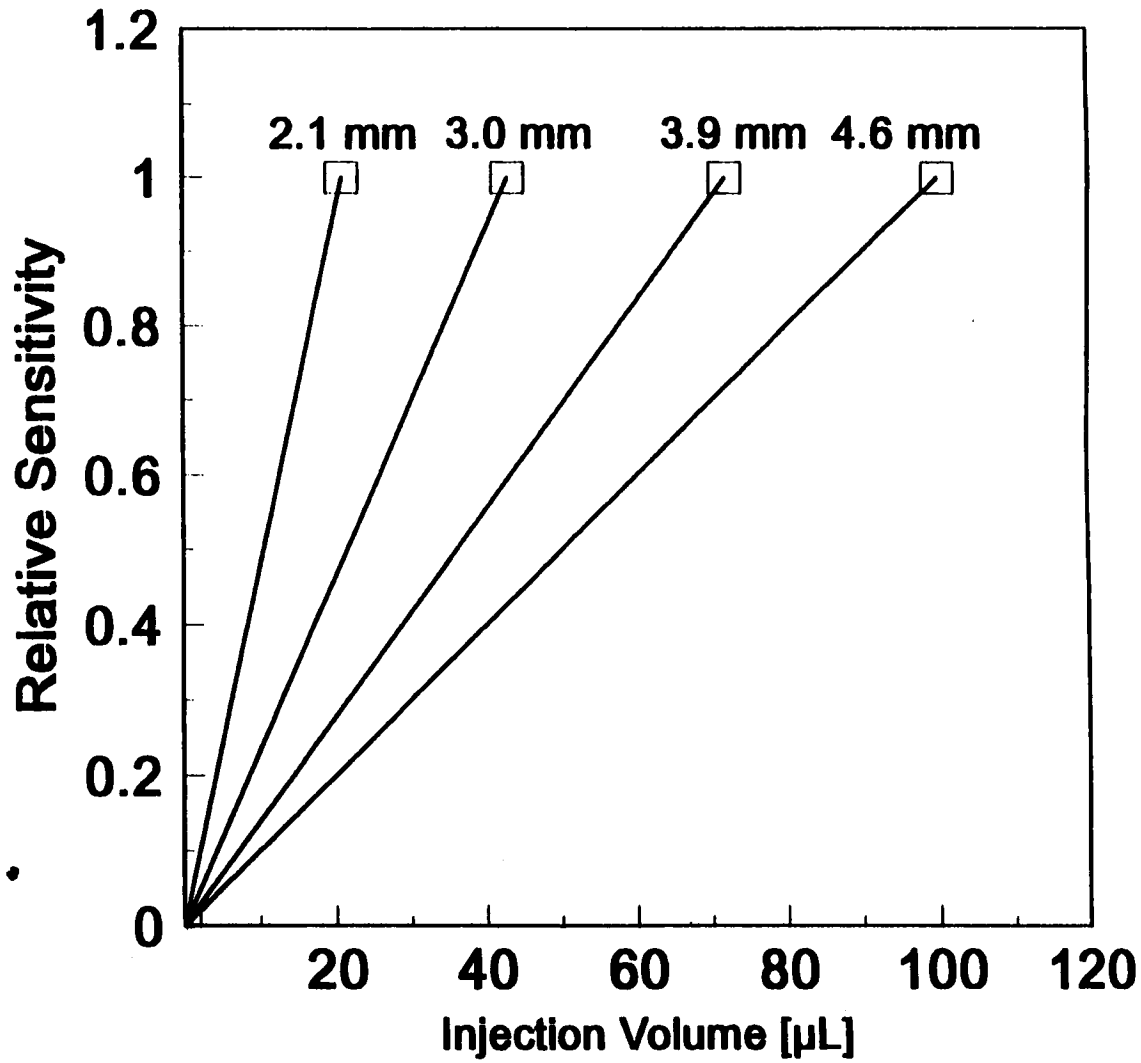
	Resolution Peaks 1 and 2	USP Tailing Peak 3
a.)	1.43	1.1
b.)	1.46	1.1
c.)	1.30	1.1
d.)	0.92	n.c.

Column Diameter and Injection Volume Effect on Detectability

- As shown on the plot of relative sensitivity versus injection volume, the same injection volume results in higher sensitivity the smaller the column diameter. An injection of 20 μl results in a relative peak height 0.2 for the 4.6 mm i.d. column and of 1 for the 2.1 mm column.
- With sufficient sample, the same sensitivity can be obtained independent of column dimensions.
- Since there is an upper limit to the amount of sample available for a given analysis, then a broad selection of column dimensions allows one to tailor the column to meet the sensitivity needs of the assay.

Effect of Column Diameter and Injection Volume on Detectability

- k = 8, N = 10,000
- L = 15 cm



Strategies in Sensitivity Enhancement

- Packing Material
  - Good Peak Shape
  - High Efficiency
- Column Dimensions
  - Smaller Diameter
  - Shorter Length
  - Smaller Particle Size

Conclusion

- Symmetry reversed-phase columns give excellent peak shapes for acidic, basic and neutral drugs independent of mobile phase pH.
- Symmetrical peaks increase sensitivity and lower the LOD and LOQ.
- Symmetry reversed-phase columns are available in four different diameters allowing one to meet the needs of the assay for resolution and sensitivity.