



# UNIQUE SELECTIVITY OF ZIRCHROM COLUMNS IN SEPARATIONS OF POLAR AND NON-POLAR MIXTURES

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## ABSTRACT

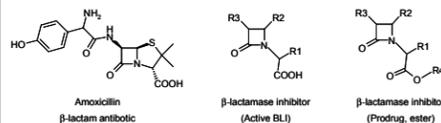
Efficient reversed-phase liquid chromatography (RP-HPLC) separations of pharmaceutical mixtures containing both highly polar and non-polar analytes are typically achieved by gradient elutions. In this work, we found that the unique selectivity observed in both ZirChrom-CARB and ZirChrom-MS columns enabled moderate retention of both polar acidic and non-polar compounds. Rapid isocratic methods were successfully developed for the separations of the highly polar acidic compound, namely amoxicillin, acidic  $\beta$ -lactam antibiotics, and the non-polar ester prodrug. More than 10 other polar columns such as polar-embedded columns and phenyl columns were also evaluated and compared. Possible separation mechanisms of ZirChrom-CARB and ZirChrom-MS columns were proposed and potential challenges were also discussed.

## INTRODUCTION

$\beta$ -lactam antibiotics are recognized with their unique structural feature – the presence of the four-member 2-azetidinone ring.



$\beta$ -lactamase inhibitors are weak antibiotics per se, but they are often used in combination with a real antibiotic agent to protect it from bacterial hydrolysis, such as amoxicillin. An acidic  $\beta$ -lactamase inhibitor (BLI) was selected as the active pharmaceutical ingredient (API), and an ester prodrug was also made to improve bioavailability. Therefore, developing an analytical method capable of analyzing all three components is required.



Both amoxicillin and the active BLI are acidic polar compounds, and they elute right at or very close to the solvent front on most reversed-phase HPLC columns.

The European Pharmacopoeia (EP) method for amoxicillin uses a 25-minute linear gradient from 92% aqueous to 80% aqueous, due to its polar characteristics. However, this method is not suitable in our application, as the non-polar prodrug needs to be eluted within a reasonable time as well.

Almost all separations of polar and non-polar mixtures rely on gradient elution. Recent advances in column technologies allow the gradient to start from 100% aqueous to increase polar components retentions. However, isocratic methods are preferred wherever it is possible because of the fast run time and robustness in method transfer.

Various column types were evaluated in this study, including polar-embedded columns, phenyl columns and zirconia columns, etc. Unique selectivity was observed in both ZirChrom-MS and ZirChrom-CARB columns, where both polar and non-polar analytes could be eluted isocratically.

## Experiments

Two gradient programs were performed on each column. With the data from gradient runs and the assistance of DryLab software, the possibility of running an isocratic method was predicted.

### Chromatographic conditions:

- System: Waters Alliance 2695 with 2996 PDA
- Mobile phases: A: 5/95/0.1 ACN/water/phosphoric acid (pH ~ 2)  
B: 70/30/0.1 ACN/water/phosphoric acid (pH ~ 2)
- Flow rate: 1.0 mL/min
- Column temp.: 35°C
- Detection: 230 nm

DryLab 2000 plus: LC Resources Inc.

Sample: Mixture of amoxicillin, active BLI and ester prodrug at 1.5 mg/mL, 1.0 mg/mL and 0.1 mg/mL, respectively.

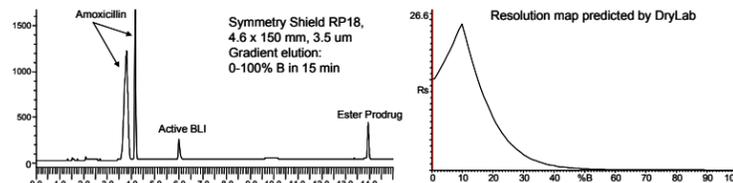
Sample diluent: 40/60/0.1 ACN/water/phosphoric acid (pH ~ 2)

## RESULTS AND DISCUSSION

### Columns Screened

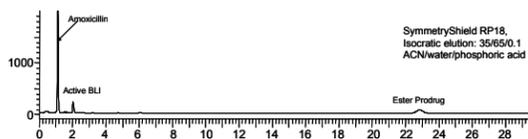
Column	Functional Group	Embedded Polar Group	Bonded Phase Chemistry	Mode of Interaction	Manufacturer
XTerra Phenyl	Phenyl	N/A		Polar, aromatic	Waters
FluoroSep RP Phenyl	Phenyl	N/A		Polar, aromatic, pi-pi electron interaction	ES Industries
SymmetryShield RP18	ODS, C18	Carbamate		Hydrophobic, polar	Waters
SymmetryShield RP8	Octyl, C8	Carbamate		Medium hydrophobic, polar	Waters
Polaris C18-A	ODS, C18	Amide		Hydrophobic, polar	MetaChem
Discovery RP Amide 16	C16	Amide		Hydrophobic, polar	Supelco
XTerra RP8	Octyl, C8	Carbamate		Medium hydrophobic, polar	Waters
AquaSep	Octyl, C8	Ether		Medium hydrophobic, polar	ES Industries
YMC Basic	Octyl, C8	N/A		Medium hydrophobic	Waters
ProTec RP18	ODS, C18	Amide*		Hydrophobic, polar, with little anion-exchange	ES Industries
Polarity RP18	Unknown	Carbamate		Hydrophobic, polar	Waters
Discovery Zr-CARB	Zirconia-Carbon	N/A		Dispersive interactions (spatial resolution); charge induced interaction (polar)	Supelco
ZirChrom-MS	Zirconia-EDTPA-PBD	N/A		Mixed mode (RP and ion-exchange)	ZirChrom Separations

### Typical Chromatograms with Polar-Embedded Column



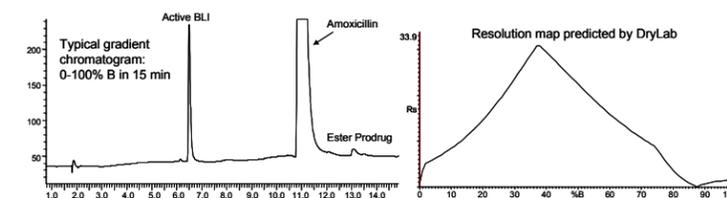
Amoxicillin appeared to be more polar than active BLI on typical polar-embedded columns.

Isocratic elution is not feasible based on DryLab prediction



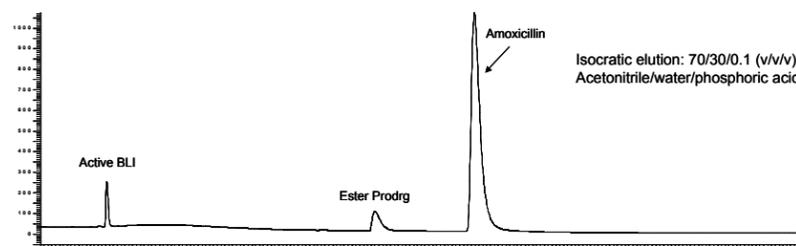
Observations: Both amoxicillin and active BLI were hardly retained under isocratic condition, while the non-polar drug had a lot of retention. It was confirmed that an isocratic condition is not applicable on typical polar-embedded columns.

### ZirChrom CARB Column



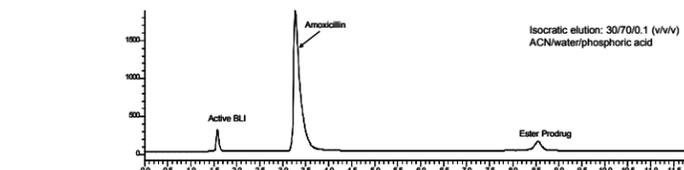
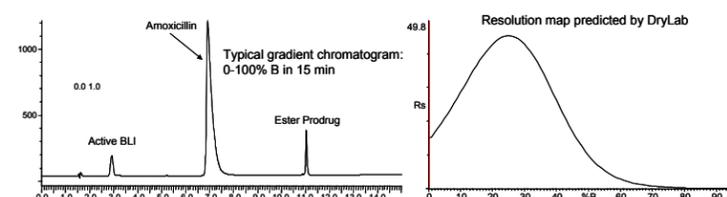
Note the behavior change of amoxicillin on ZirChrom CARB column.

Isocratic elution is possible based on DryLab prediction.



Note: The elution order of the three components is different from typical polar-embedded columns, where amoxicillin had even more retention than the ester prodrug.

### ZirChrom MS Column



Observations: Elution order different from both ZirChrom-CARB and typical polar-embedded columns was observed. Isocratic elution is possible on ZirChrom-MS column.

## Possible Separation Mechanisms

### ZirChrom-CARB:

ZirChrom-CARB column uses zirconia as the substrate. The non-polar stationary phase is pyrolytic carbon coated onto the solid support. At low pHs (<4), the column shows net positive charge; therefore, mix-mode separation mechanism-hydrophobic interaction and anion-exchange was in effect. In addition, the column also retains analytes through electronic ( $\pi$ - $\pi$ ) interactions\*, which might account for the strong retention observed on amoxicillin over the active BLI.

### ZirChrom-MS:

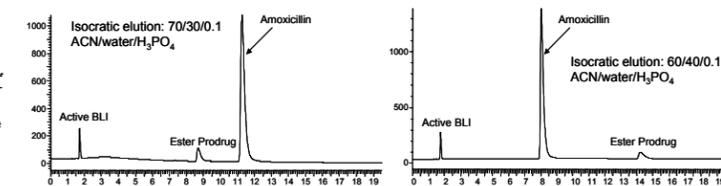
ZirChrom-MS phase is Lewis acid site deactivated. It shows net zero charge at low pHs and appears to have net-negative charge at pH 5 and above. Therefore, it offers mix-mode separation mechanism and unique selectivity for basic compounds. Interestingly, it also shows different selectivity from both ODS and ZirChrom-CARB columns. It is much less retentive for the non-polar compound compared to ODS column in this application, but also provides reasonable retention for some acidic analyte (e.g., amoxicillin).

\* T.P. Weber, P.T. Jackson and P.W. Carr, *Analytical Chemistry*, 1995 (67), 3042-3050

## Potential Challenges

### ZirChrom-CARB:

Method robustness is often a challenge for mix-mode columns. The same issue is also revealed from the DryLab resolution map, as small changes in B% would generate large changes in both resolution and run time. Reverse of elution order was also predicted by DryLab, which was later confirmed with experiments (as seen below). Similar phenomena were also observed on another mix-mode column – ProTec RP18.



### ZirChrom-MS:

ZirChrom-MS column exhibits better method robustness for this particular application compared with the CARB column, which was consistent with the DryLab prediction. As for neutral and acidic compounds at low pHs, no ion-exchange mechanism was present.

## CONCLUSION

Rapid isocratic methods were developed for a mixture of polar and non-polar compounds on both ZirChrom-CARB and ZirChrom-MS columns, where similar separation could only be achieved by gradient elution on conventional ODS or polar-embedded columns. Different retention mechanisms were exhibited for the two columns. At mix-mode separation, retention of amoxicillin was very sensitive to changes in mobile phase composition. Better method robustness was observed on ZirChrom-MS column.

## ACKNOWLEDGMENT

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