Fast Analysis of Small MW Analytes in a Beverage and Serum Samples on a Zirconia-based Strong Anion-Exchanger

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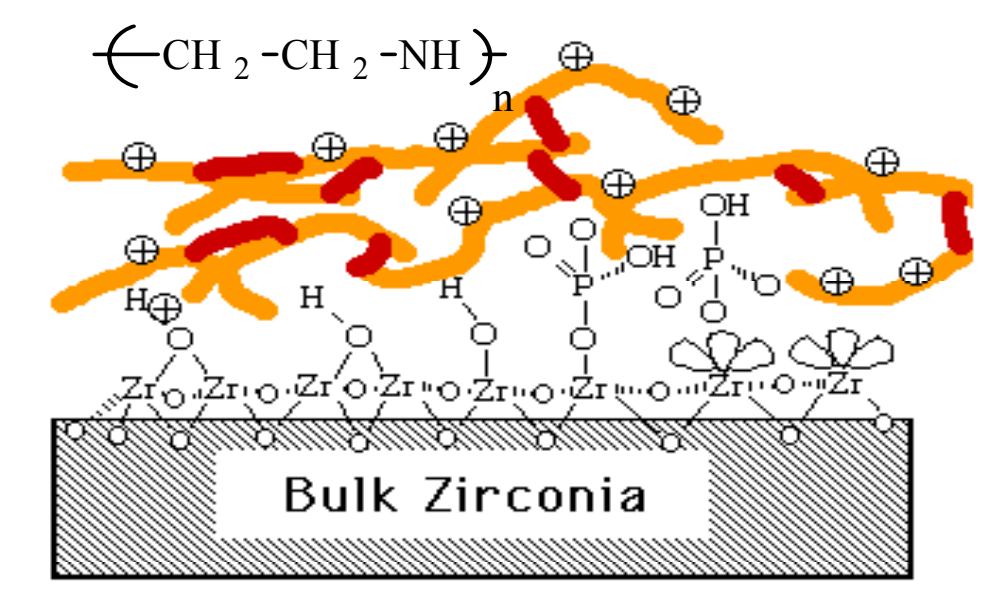
Abstract

The zirconia-based anion-exchange phase, sold commercially as ZirChrom®-SAX, is an anion-exchange material that is built on microporous zirconia and contains a substantial amount of hydrophobic cross-linker that imparts mixed-mode ion-exchange/reversed-phase characteristics. Here we demonstrate the "green" analysis of several commercial diet soft drinks in combination with a 100% water mobile phase. The separation of caffeine, aspartame, and benzoate shown here in a 100% aqueous mobile phase has the capability of the mixed-mode column to separate these compounds of wide-ranging chemical properties. The 100% aqueous mobile phase containing dilute amounts of phosphate and carbonate is completely environmentally friendly, eliminating the need for costly handling and disposal of the eluent. Finally, the thermal stability of the ZirChrom®-SAX material allows the separation to be carried out at 50 °C to decrease overall analysis time. Also shown is a fast, sensitive and accurate detection and quantitation method for vitamin C in plasma samples using ZirChrom®-SAX and a ZirChrom®-Protain column to remove matrix-proteins on-line by direct sample injection. The method requires no additional sample preparation thereby reducing the cost of analysis compared to more traditional techniques that firs require a sample preparation step.

Surface Chemistry and Retention Mechanisms of QPEI-Zirconia

- **Anion-exchange**
- **Hydrophobic interactions**
- **Lewis acid-base interactions**



Ion Exchange Mechanism Study on ZirChrom-SAX

$$\log k' = C - \frac{x}{y}\log\left[E_m^{y-}\right]$$

Columns, 5 cm x 0.46 cm I.D.; mobile phase, A: 0.02 M potassium phosphate dibasic, B: 0.02 M potassium phosphate dibasic0.75 M NaCl, adjusted to pH 7 with HCl; 0.08-0.56M NaCl; flow rate, 1.0 ml/min; injection volume, 20 μl; detection at 220nm.

	Slope		
Column	$(Asp)_2$	$(Asp)_3$	$(Asp)_4$
A0.5-5	2.32	2.22	3.07
A2-5	2.3	2.53	3.09
A5-5	2.17	2.56	3.49
A10-5	2.19	2.63	3.47

	R^2		
Column	$(Asp)_2$	$(Asp)_3$	$(Asp)_4$
A0.5-5	0.992	0.999	0.999
A2-5	0.930	0.985	0.997
A5-5	0.974	0.990	0.998
A10-5	0.959	0.989	0.998

Study of Hydrophobic Interactions on QPEI-Zirconia

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Experimental Conditions

- p-Alkoxybenzoic acids solutes
- 0.10M k_2HPO_4, 0.50M NaCl @ pH7
- 1 ml/min, 35°C, UV-240 nm

Free Energy of Transfer

- $\Delta G^o = -RT \ln (k'/\phi)$
- $\Delta G^o retn,CH_2 = -RT \ln (k'_{n+1}/k'_n)$
- Typical C_{18} = -2.4 KJ/mole

Column	Slope	Intercept	R^2	ΔG^o_{CH2} (KJ/mole)
A0.5-5	0.786	0.654	0.998	-2.01
A2-5	0.705	0.898	0.996	-1.81
A5-5	0.703	0.073	0.998	-1.80
A10-5	0.720	0.050	0.998	-1.84

Principal Components Analysis of Capacity Factors on ZirChrom-SAX

- 4 SAX phases
- 34 solutes
- 5 mobile phases: no blocking agent, 0.1M phosphate, 0.1M fluoride, 0.1M fluoride + 10% methanol, fluoride at 30 and 60^{o}C, 0.001M NaF after flushing
- Data mean-centered and normalized
- 34 x 20 data matrix

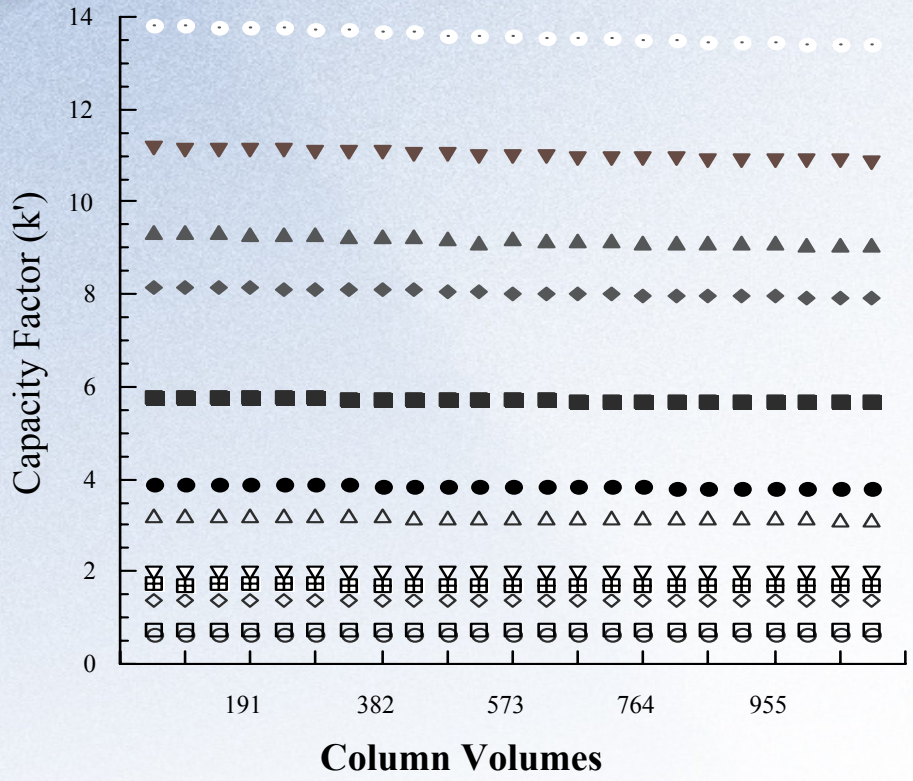
Principal Components Analysis of lnk′ Data on ZirChrom-SAX

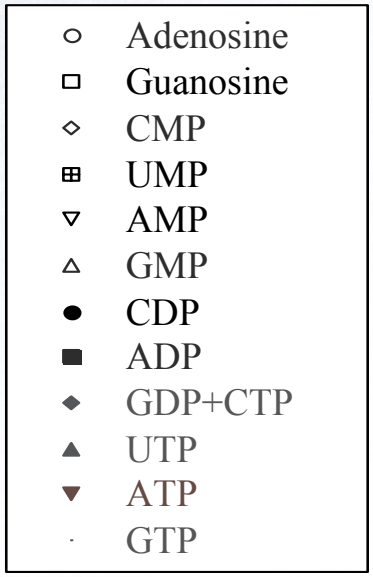
Factors	% Variance	Total % Variance
1	95.35	95.35
2	3.403	98.75
3	0.727	99.48
4	0.163	99.64
5	0.132	99.78

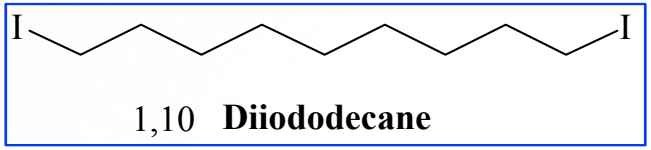
- **Conclusion:**

  There are ***three principal components*** that are needed to account for 99.5% of the variance in the data.

Stability of ZirChrom-SAX at a Column Temperature of 75°C







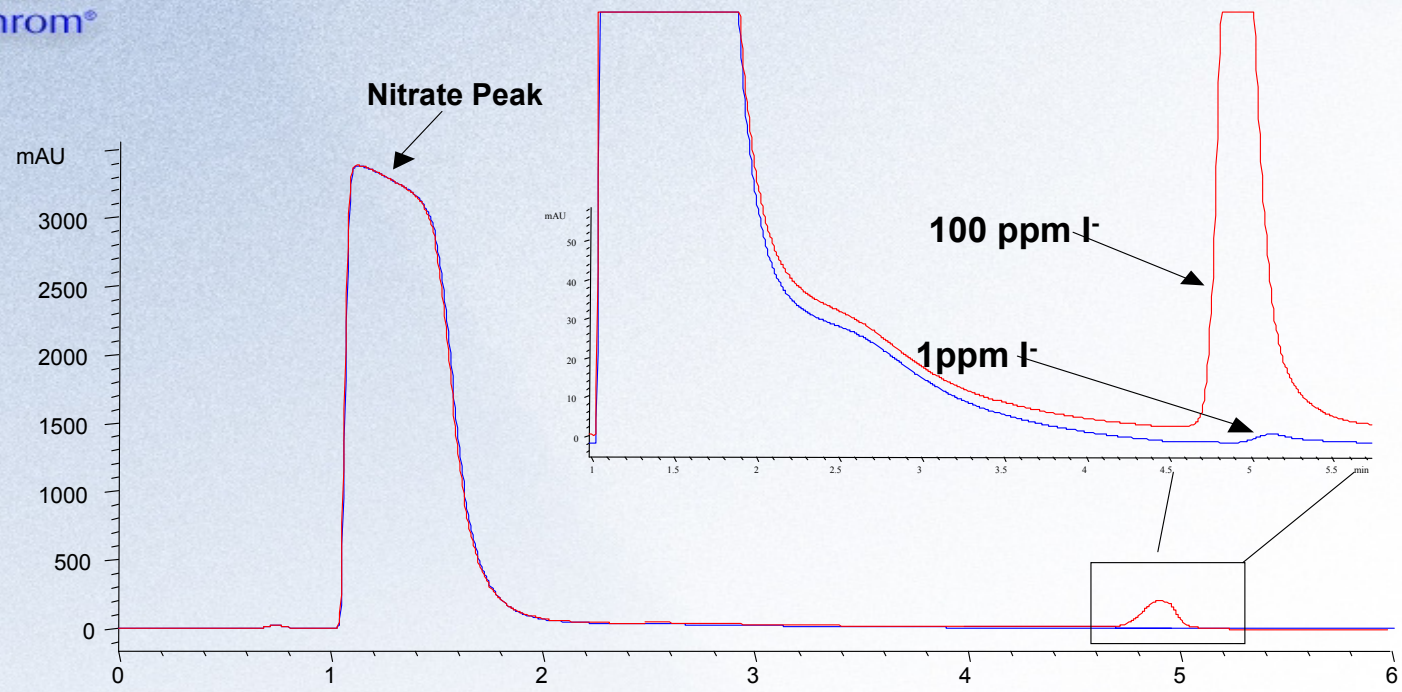
mobile phase, A =0.01M potassium phosphate dibasic, 0.04M NaCl @ pH 7.0, B = ; 0.1M potassium phosphate dibasic, 0.4M NaCl @ pH 7.0; gradient, 10% B to 90% B over 25min; injection volume, 5 μl; column temperature, 75°C; UV detection @ 240 nm

Comparison of ZirChrom-SAX to a SAX-Silica Column

Solute	h (silica-SAX)	h (QPEI ZrO_2 A2-5)
bromate	3.4	3.7
nitrite	3.3	4.2
nitrate	4.4	4.3
iodide	3.0	3.7
benzoic acid	14.3	4.9
p-amino BA	5.3	6.5
p-hydroxy BA	8.3	6.8
p-cyano BA	13.9	5.4
p-fluoro BA	19.0	5.2
p-isopropyl BA	11.8	8.2
p-nitro BA	12.6	5.8
p-chloro BA	10.9	5.8
p-bromo BA	12.9	5.9
p-iodo BA	13.8	6.5
average	9.8	5.5

Silica SAX = silica-based strong anion exchanger by Machnerey Nagel
5 μm, 350 m^2/g; mobile phase, 0.1M phosphate + 0.4 M NaCl @ pH 7.4

Trace Iodide Separation on ZirChrom®-SAX

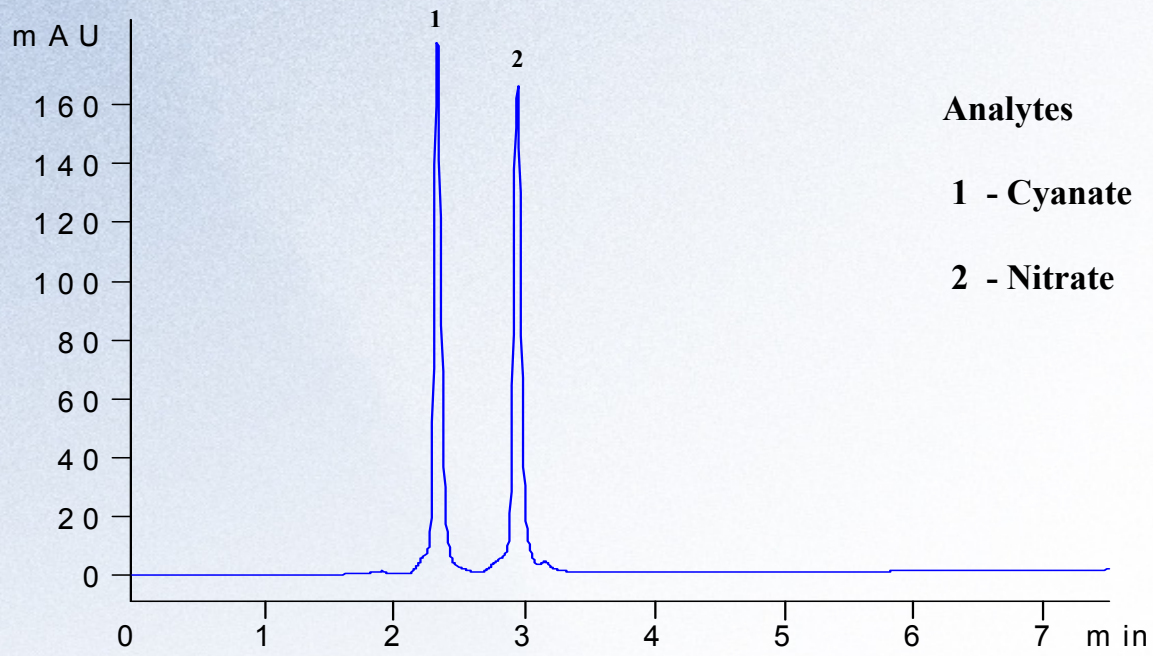


Analytes

2M nitrate sample matrix and Iodide

LC Conditions: Column: ZirChrom®-SAX, 50 x 4.6 mm, Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 226 nm, Injection volume: 5 ul, Mobile Phase: 25mM ammonium phosphate, 275mM NaCl at pH 8.0

Fertilizer Plant Effluent Ions on ZirChrom®-SAX



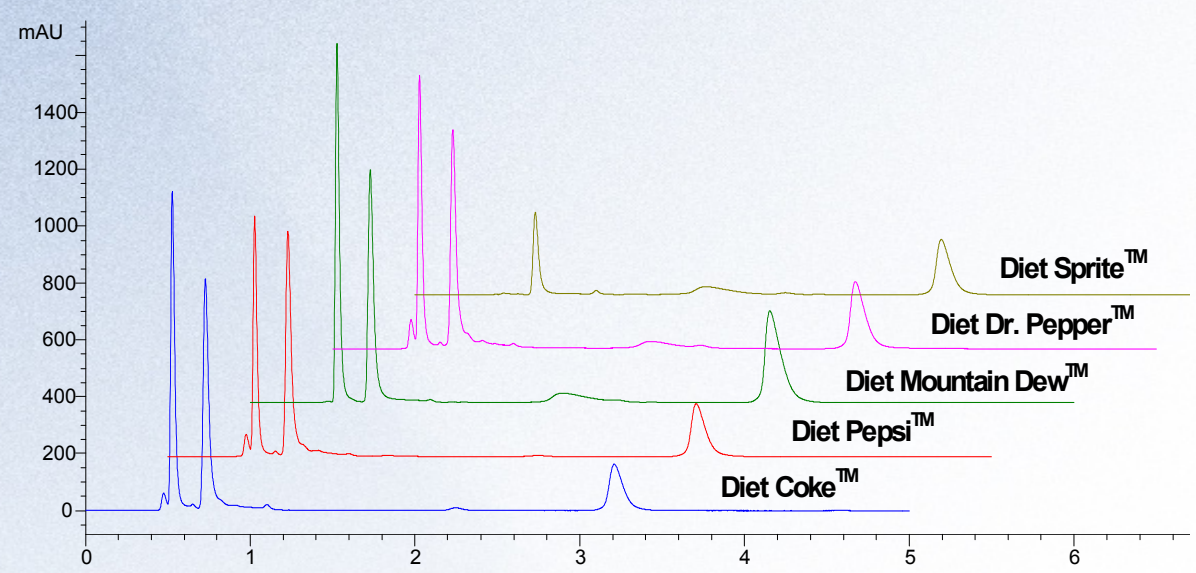
Analytes

1 - Cyanate (1.0 mg/ml)

2 - Nitrate (.02 mg/ml)

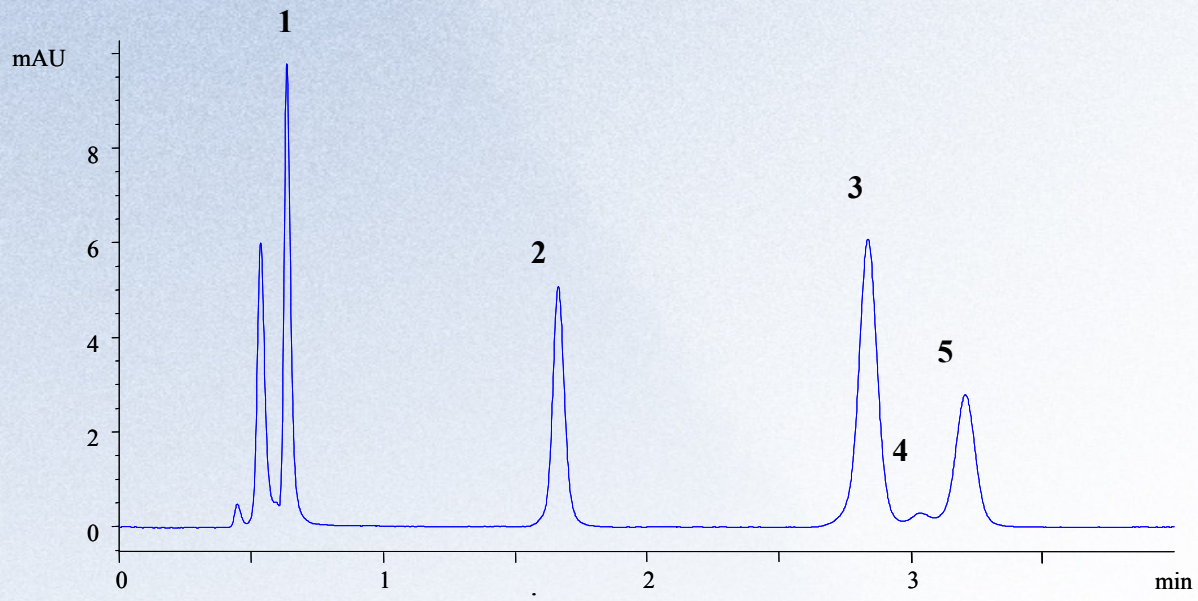
LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm, Flow rate: 1.0 mL/min. Temperature: 30°C, Detection: 205 nm, Injection volume: 5 ul, Mobile Phase: 25mM sodium fluoride, 175mM sodium chloride at pH 10.0

"Green" Analysis of Diet Soft Drinks Containing Caffeine and Aspartame



LC Conditions: Column: 3.0 mm x 100 mm ZirChrom®-SAX, Mobile Phase: 10mM Ammonium phosphate, 5mM Ammonium carbonate, pH 6.6, Flow rate: 1.0 ml/min, Temperature: 50 °C, Injection Vol.: 5.0 µl, Pressure Drop: 205 bar Detection: UV at 210 nm.

LC/MS Compatible Separation of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) on ZirChrom®-SAX



1-Acetaminophen

2-Ibuprofen

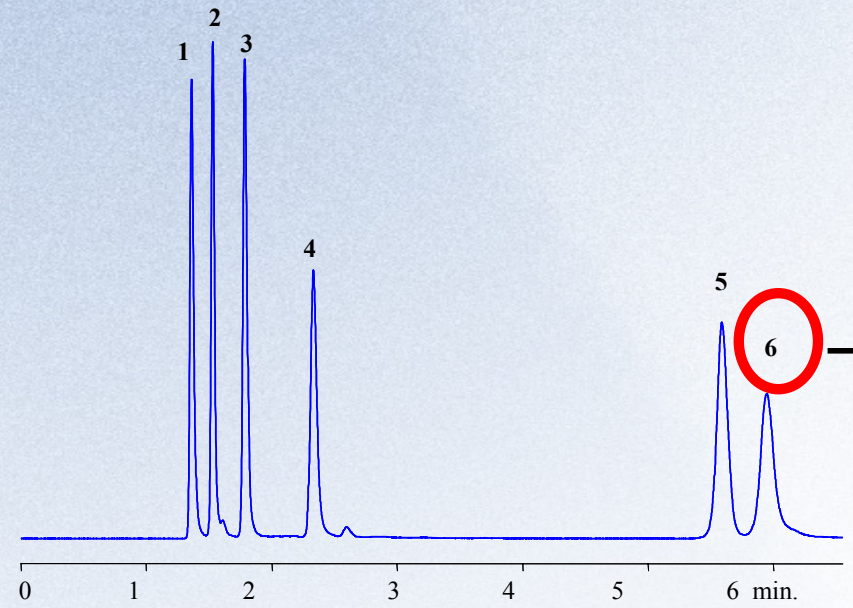
3-Naproxen

4-Impurity

5-Ketoprofen

LC Conditions: Column: ZirChrom®-SAX, 50 x 4.6 mm i.d. (part number: ZR06-0546), Mobile Phase: 80/20 ACN/15 mM ammonium formate, pH=4.0 (adjusted with formic acid), Flow rate: 1.0 ml/min. Temperature: 35 °C, Injection Vol.: 1.0 µl, Detection: UV at 254 nm

Water-Soluble Vitamin Analysis on ZirChrom®-SAX



1 - Thiamine (Vit. B_1)
2 - Pyridoxine (Vit. B_6)
3 - Nicotinamide (form of Vit. B_3)
4 - Riboflavin (Vit. B_2)
5 - Nicotinic acid (form of Vit. B_3),
6 - Ascorbic acid (Vit. C)

Vitamin C is largely retained on ZirChrom-SAX

LC Conditions: Column: ZirChrom®-SAX, 150 x 4.6 mm i.d. (part number: ZR06-1546), Mobile Phase: 50 mM Ammonium dihydrogenphosphate, pH 4.5, Flow rate: 1.0 ml/min. Temperature: 30 °C, Injection Vol.: 5.0 µl, Detection: UV at 254 nm

Conclusions

- ZirChrom® -SAX is an *alkali, acid and temperature* stable stationary phase useful for anion-exchange chromatography.
- ZirChrom® -SAX has *unique selectivity* in the separation of bio-molecules due to *"mixed-mode"* separations that include hydrophobic, electrostatic and Lewis acid-base interactions.
- ZirChrom® -SAX can be used for ion-exchange *LC-MS* using *volatile* mobile phases.

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