Application Note

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Chiral Analysis of Sodium Lactate with Agilent InfinityLab Poroshell 120 Chiral-T Columns

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Introduction

Faster separations of enantiomers have become increasingly important in biochemical analysis and the pharmaceutical industry. As increasing numbers of optically active medicinal compounds are introduced, along with increasing government regulation, it is important that rapid, sensitive, and reliable methods be devised for their analysis. Over half of the drugs currently in use are chiral compounds, and near 90% of the last ones are marketed as racemates, consisting of an equimolar mixture of two enantiomers. Although they have the same chemical structure, most isomers of chiral drugs exhibit marked differences in biological activities.

While many chiral separations are carried out using cellulose or amylose-based chiral selection phases (CSP) and use normal phase solvents like hexane, other phases are frequently sought for separation based on more common solvents like methanol. These can be more easily incorporated into a laboratory running reversed phase methods.

Glycopeptide-based chiral columns, such as the Agilent InfinityLab Poroshell 120 Chiral-T (Teicoplanin) and Agilent InfinityLab Poroshell 120 Chiral-V (Vancomycin) can be used in a wide variety of solvents in reversed-phase and normal phase high-performance liquid chromatography (HPLC), as well as supercritical fluid chromatography (SFC). Glycopeptides are amphoteric, containing both ionizable acidic and basic groups. Thus, they can be positively charged, negatively charged, or neutral, depending on the pH of the mobile phase. This allows for ionic interactions involved in chiral recognition when separating ionic compounds using this class of CSP. This is thought to play a major role in chiral recognition for this class of CSPs. Other possible interactions involved with the use of antibiotics as CSPs for chiral recognition include hydrogen bonding, steric, dipole–dipole, and π–π interactions, as well as hydrophobic interactions. These interactions may take place in different combinations that are determined by the properties of an individual analyte and the mobile-phase mode used. Each separation mode provides simultaneous, but different interactions for chiral recognition. This accounts for the large number of chiral separations and the variety of types of chiral compounds that are successfully separated with this class of CSP.

The glycopeptide Teicoplanin is covalently bonded to superficially porous silica particles, creating stable and solvent-resistant chromatographic media. These covalently-bonded phases are resistant to common HPLC mobile phases and additives, such as methanol, ethanol, IPA, THF, phosphate, formate, acetate, formic acid, TFA, TEA, and NH_4OH. In this application note, a separation for sodium lactate as lactic acid is developed and optimized on the InfinityLab Poroshell 120 Chiral-T.

Experimental

An Agilent 1290 Infinity LC configured for low dispersion was used for this work. Table 1 shows the instrument configuration. All compounds were injected as mixtures of enantiomers and as individual standards for identification.

Sodium D,L-lactate and sodium L-lactate were purchased from Sigma-Aldrich. The L enantiomer was dissolved at 1 mg/mL, and the mixed D,L enantiomer was dissolved sample in methanol at 2 mg/mL. The structure of sodium lactate is shown in Figure 1. Ammonium acetate and ammonium formate (LC/MS grade) were also purchased from Sigma Aldrich. HPLC-grade methanol was purchased from Honeywell.

Mobile phases were prepared at 0.2% w:v by dissolving 2 g of ammonium formate or ammonium acetate in methanol. Lower concentrations were also prepared in a similar manner.

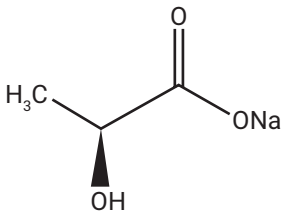

Figure 1. Structure of sodium lactate.

Results and discussion

In general, good separations of acidic chiral compounds (such as amino acids and other compounds) are documented on the InfinityLab Poroshell 120 Chiral-T column. In this work, an initial screening with an InfinityLab Poroshell 120 Chiral-T and three mobile phases was carried out.

1. MeOH/20 mM ammonium formate, pH 4.0 90/10 RP
2. MeOH/20 mM ammonium formate, pH 4.0 30/70 RP
3. MeOH with a 0.2% w:v ammonium formate mobile phase

Table 1. Instrument configuration and analysis conditions.

1290 Infinity LC System	
Agilent 1290 Flexible Pump (G7104A)	
Agilent High Performance Autosampler (G4226A)	– Seat assembly, ultralow dispersion, for Agilent 1290 Infinity autosampler G4226A (p/n 4226-87030) – Autosampler and heater: capillary, stainless steel, 0.075 × 220 mm (p/n 5067-4784) – Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) – Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent Multicolumn Thermostat (MCT) (G7116B)	– Ultralow dispersion heater p/n 7116-60021 – Heater and column: InfinityLab Quick Connect assembly, 105 mm, 0.075 mm (p/n 5067-5961) – Column and ELSD capillary, stainless steel, 0.075 × 220 mm, SV/SLV (p/n 5067-4784)
Agilent 1290 ELSD II (G7102A)	– Evaporator temperature 30 °C – Nebulizer temperature 30 °C – Gas flow rate 1 SLM – 40 Hz
Agilent OpenLab CDS, version C.01.07	

The initial screening using 0.2% ammonium formate showed some success with the sodium L lactate, but the chromatogram of the D,L-lactate showed poor peak shape (Figure 2). In a second experiment, ammonium formate was changed to 0.2% ammonium acetate. This yielded excellent peak shape with good resolution of all three peaks (Figure 3). An attempt at optimization was made by lowering the ammonium acetate concentration, however, the retention time of the last peak substantially increased, while not substantially increasing resolution (Figure 4). This last peak was identified as sodium, by injecting a sample of sodium chloride (Figure 5). The mode of separation is described as polar ionic separation. Polar ionic separations typically involve the use of organic solvents such as methanol or acetonitrile, with small amounts of acid and base added. Optimization of the separation is frequently accomplished by varying the acid base ratio using acetic acid/triethylamine or ammonium hydroxide. Alternatively, an ammonium salt is added to the mobile phase (ammonium formate, ammonium acetate, or ammonium trifluoroacetate). The mobile phase typically used in screening is 0.2% w:v ammonium formate, and is used with the InfinityLab Poroshell 120 Chiral-V (Vancomycin) or InfinityLab Poroshell 120 Chiral-T (Teicoplanin).

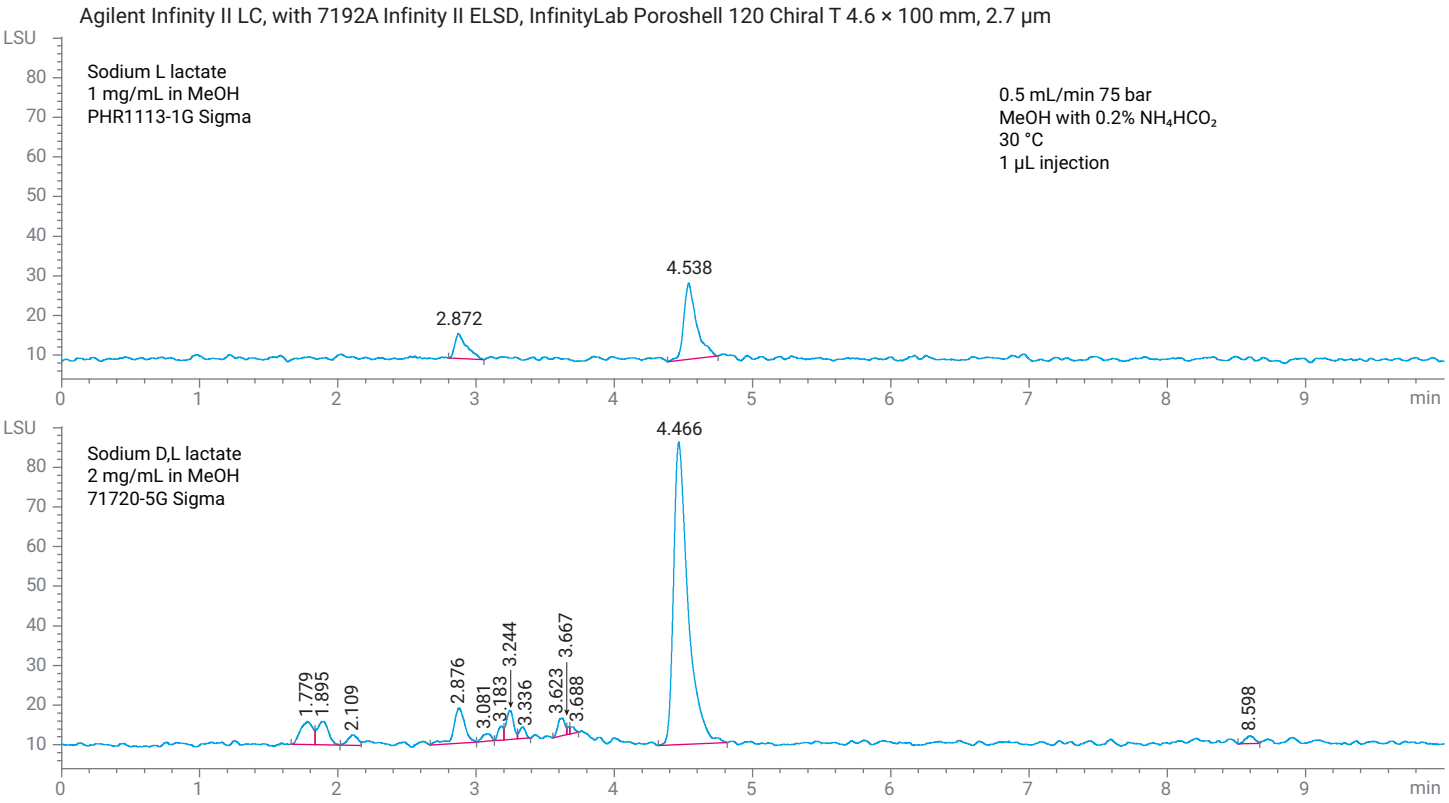

Figure 2. Separation of sodium D,L-lactate in 0.2% ammonium formate.

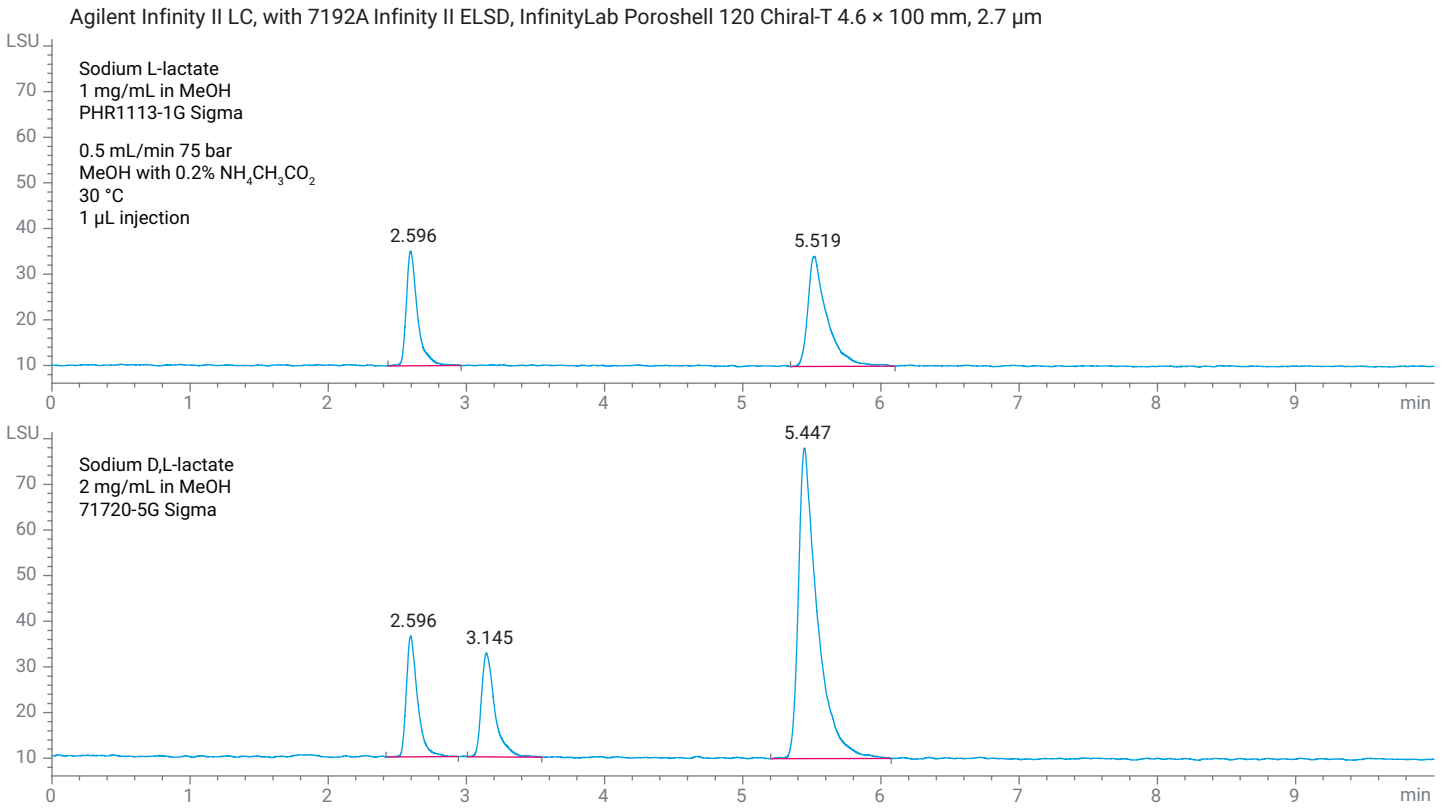

Figure 3. Separation of sodium D,L-lactate in 0.2% ammonium acetate.

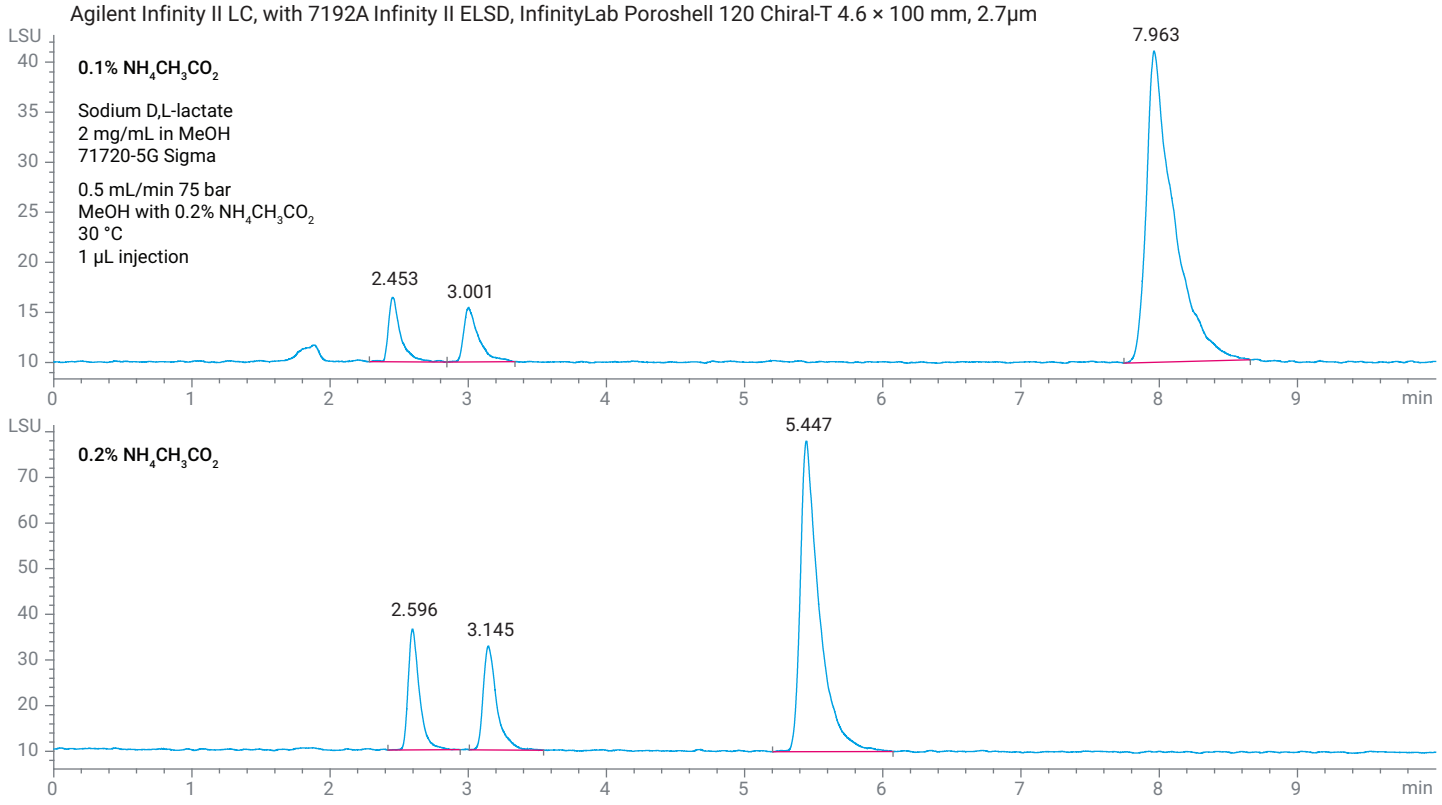

Figure 4. Evaluation of ammonium acetate concentrations.

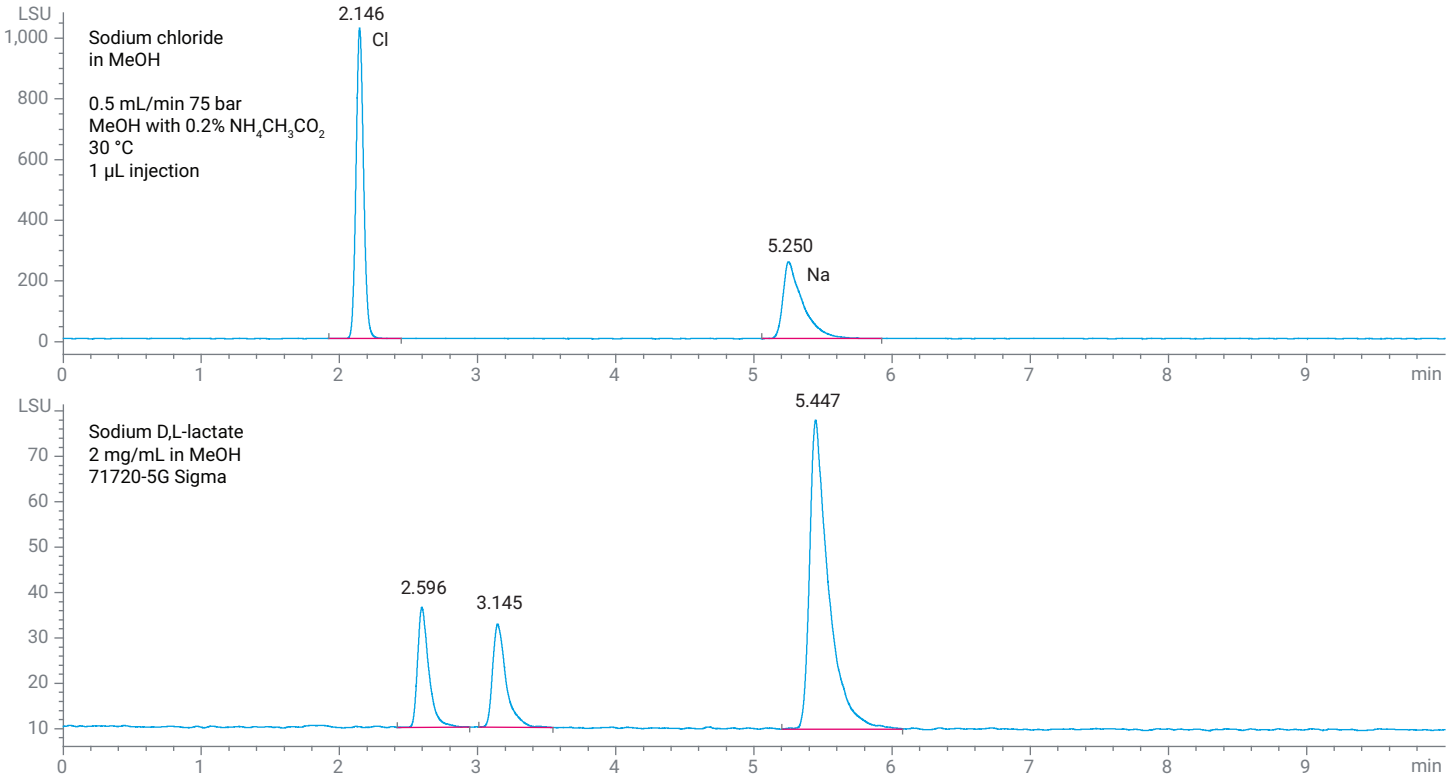

Figure 5. Identification of sodium and lactate enantiomer peaks.

Conclusion

The Agilent InfinityLab Poroshell 120 Chiral-T column supports a robust method for the separation of sodium lactate. This column offers good resolution and peak shape for all compounds studied and is compatible with mass spectrometric analysis.

References

1. Bonner, W. A. Parity Violation and the Evolution of Biomolecular Homochirality. *Chirality* **2000**, 114.
2. Put InfinityLab Poroshell 120 Chiral Innovation to Work for Your Challenging Separations. *Agilent Technologies applications compendium*, publication number 5991-8450EN, **2017**.

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