

## **LC - Instrument Parameters Bile Acid Kit (Biocrates Life Science AG, Innsbruck, Austria)**

### **System:**

UPLC (Waters Acquity, Waters Corporation, Milford, USA)

### **Column:**

UPLC column available from Biocrates (NProduct-Number: 91220051220868)

### **Precolumn:**

Security Guard ULTRA Cartridge C18/XB-C18 for 2.1mm ID column, Phenomenex (AJ0-8762)

### **Materials:**

Water MiliQ,

Methanol (Merck KGaA, Darmstadt, Germany, hypergrade for LC-MS)

Acetonitril (Merck KGaA, Darmstadt, Germany hypergrade for LC-MS)

Ammonium acetate (Honeywell - Fluka, Seelze, Germany)

Formic acid (Honeywell, Fluka, Seelze, Germany)

Stock Solution: 200mM Ammonium acetate in MiliQ Water

A: 25mL stock solution+475 mL MilliQ water + 75µL fresh formic acid

B: 25mL stock solution + 150mL methanol + 325mL acetonitrile + 75 mL fresh formic acid

### **Method**

#### **Gradient Table**

Time (min)	Flow Rate(ml/min)	%A	%B	Curve
Initial	0.500	65.0	35.0	Initial
0.25	0.500	65.0	35.0	6
0.35	0.500	60.0	40.0	6
1.90	0.800	55.0	45.0	6
2.10	0.800	45.0	55.0	6
3.30	1.000	35.0	65.0	6
3.50	1.000	0.0	100.0	6
4.00	1.000	0.0	100.0	6
4.10	0.900	65.0	35.0	6
5.00	0.500	65.0	35.0	6