URINARY CATECHOLAMINES

This memo describes some suggestions for a XLC method for water samples containing the following compounds:

- Noradrenalin (NA)
- Adrenalin (A)
- Dihydroxylbenzilamine (DHBA, IS)
- Dopamine (DA).

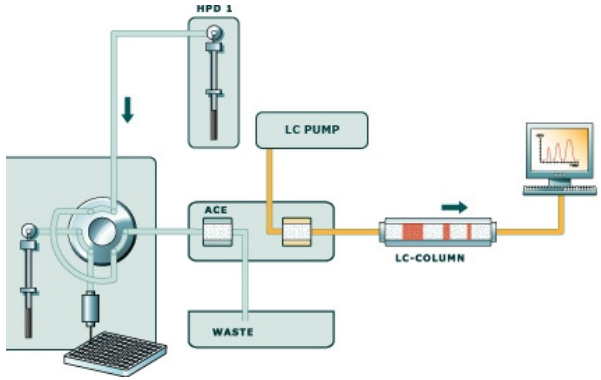


Figure 1: Schematic representation of the flow path in the Symbiosis System.

Sample pre-treatment

Sample was diluted in twofold with 2 M ammonia-ammonium chloride buffer pH 8.5, containing 0.5% EDTA then it was complexed with an equal volume of 2 M ammonia-ammonium chloride buffer pH 8.5, containing 0.5% EDTA (solvent A) and 0.2% diphenylborate (PBA). To 1.2 mL of this mixture 20 µL internal standard solution (1.26 g/L dihydroxybenzylamine) was added.

Injector conditions

Injection 200 µL

Table 1: Autosampler settings

SPE conditions

Cartridge:	10x2 mm PLRPS (0722.301)	
Solvation:	1 mL Methanol	1.0 mL/min
Equilibration:	0.5 mL water and 10x diluted solvent A (solvent B)	1.0 mL/min
Sample Load:	1 mL solvent B	1.0 mL/min
Wash:	1 mL solvent B/methanol (80/20) and 2.15ml water	1.0 mL/min
Elution	0.5 min elution of columns 1 & 2 with mobile phase	1.0 mL/min
Elution	Switch column 1 to waste 1.15 min after elution	1.0 mL/min

Table 2: SPE settings

LC conditions

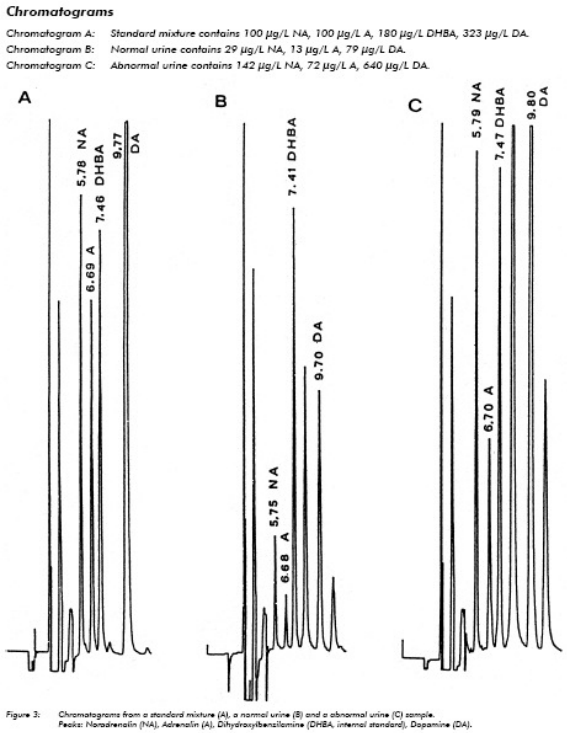
Column 1:	C18, 30 x 4.6 mm, 5 µm (Brownlee Labs, Applied Biosystems)
Column 2:	C18, 250 x 4.6 mm, 5 µm (Beckman Instruments)
Mobile phase:	50 mMol potassium dihydrogen phosphate, 1 mMol sodium heptane sulfate and 0.07 mMol EDTA solution / methanol / acetonitril (v/v 100:8:15). Final pH was adjusted to 3.2 with 1.5 M orthophosporic acid.
Flow rate	0.8 mL/min
Run time	18.30 min.

Table 3: LC conditions

Detector conditions

ESA	Oxidizing electrode: +350 mV
Coulachem	Screen electrode: +100 mV
dual cell	Quantifying electrode: 300 mV

Table 4: Detector conditions



Reference:

- Spark Holland Application Info 42

The method described in this application memo is meant as a generic starting point and may need to be optimized depending on actual experimental conditions.