Catecholamine Analysis with Star 9080 EC Detector

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Varian Application Note
Number 16

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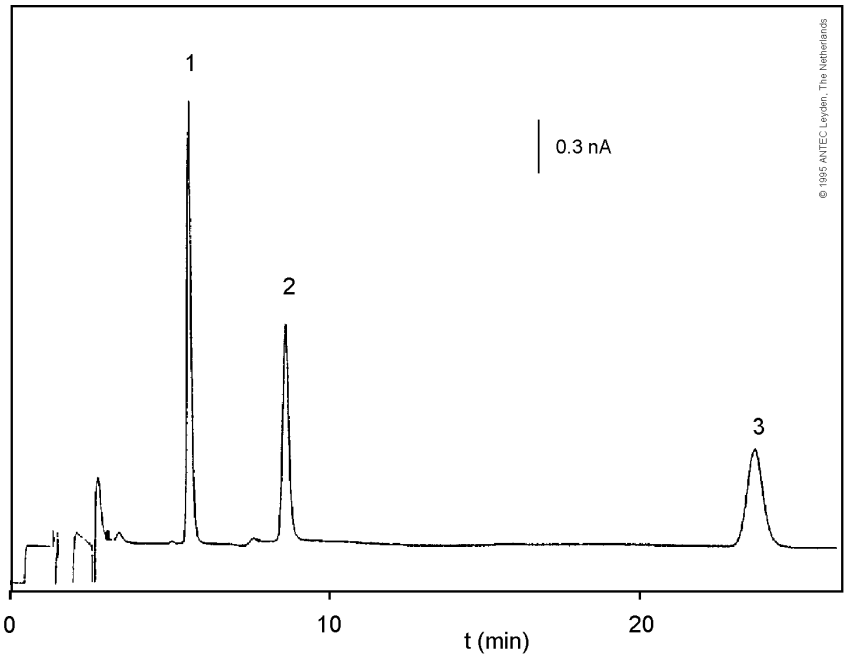
Key Words: *Star 9080, Catecholamines, Clinical, Biomedical*

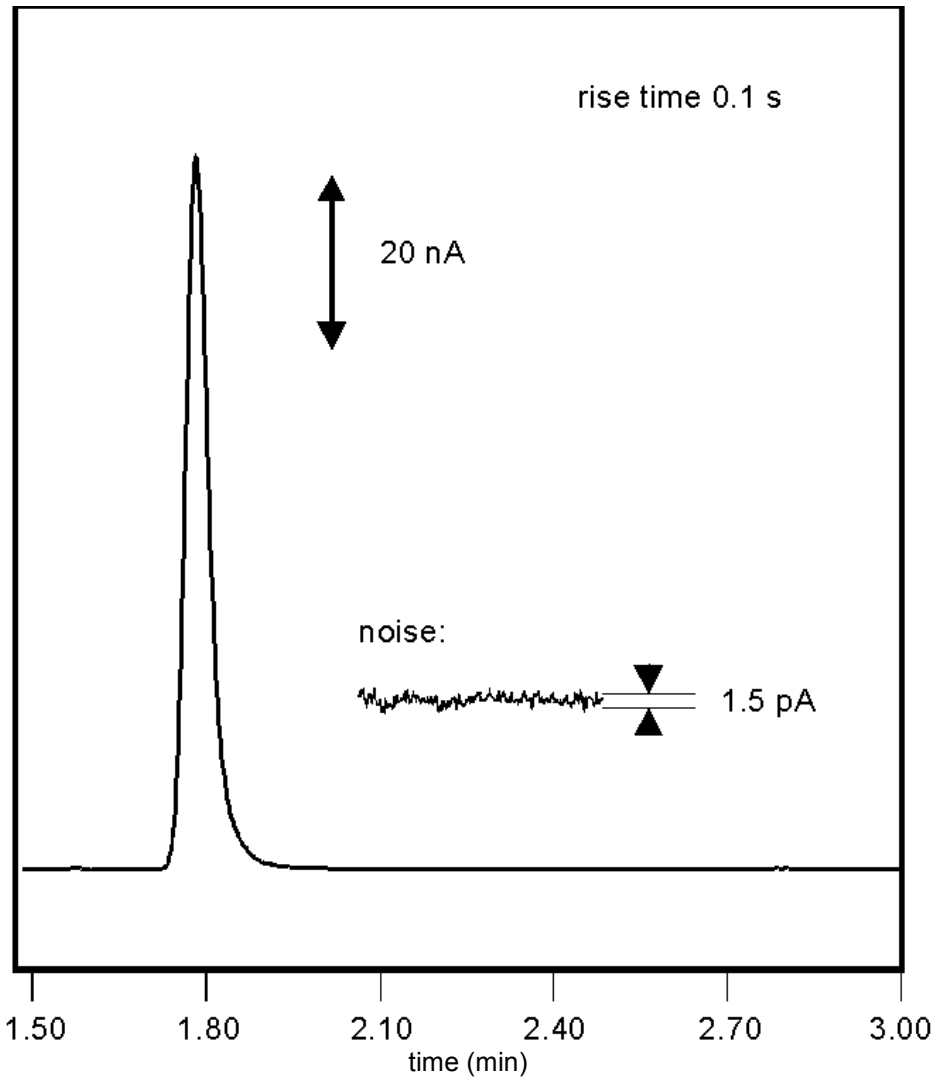
Standard Separation



Analysis of 30 nmol/L noradrenaline (1), adrenaline (2) and dopamine (3).

Detector	Varian Star 9080 Amperometric Electrochemical Detector
Column	ODS, 4 µ, 150 x 4.6 mm
Flow rate	1.0 mL/min
Mobile phase	H_3PO_4 50 mM, citric acid 50 mM, pH=3.1 with KOH, 20 mg/L EDTA, 100 mg/L octane sulphonic acid (OSA), 5% methanol
Sample	20 µL injection catecholamine standards, 30 nmol/L
Temperature	30 °C
Flowcell	2.74 mm Glassy Carbon working electrode
REF	Ag/AgCl reference electrode filled with saturated KCl/AgCl
E-cell	800 mV (vs. Ag/AgCl)
I-cell	3 nA (background current)

Calculation of S/N Ratio and Detection Limit



A typical S/N ratio for norepinephrine measured with a VT-03 glassy carbon flowcell is 53000.
The amount injected is 20 pmole (1.0 µmol/L).
The concentration detection limit based on three times the noise (pp) is 56 pmol/l.

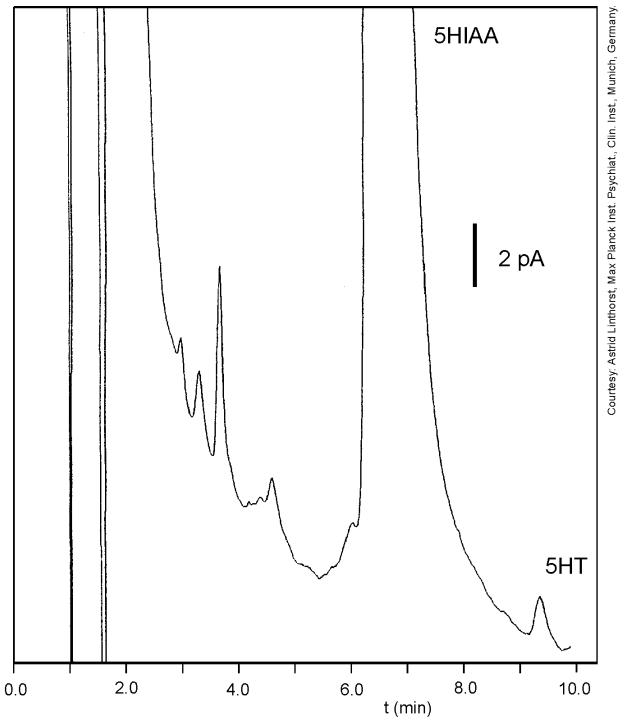
Calculations:

$$\text{S / N ratio} = \frac{\text{signal (nA)}}{\text{noise pp (nA)}} = 53000 \text{ (1.0 µmol/L, 20 µL)}$$

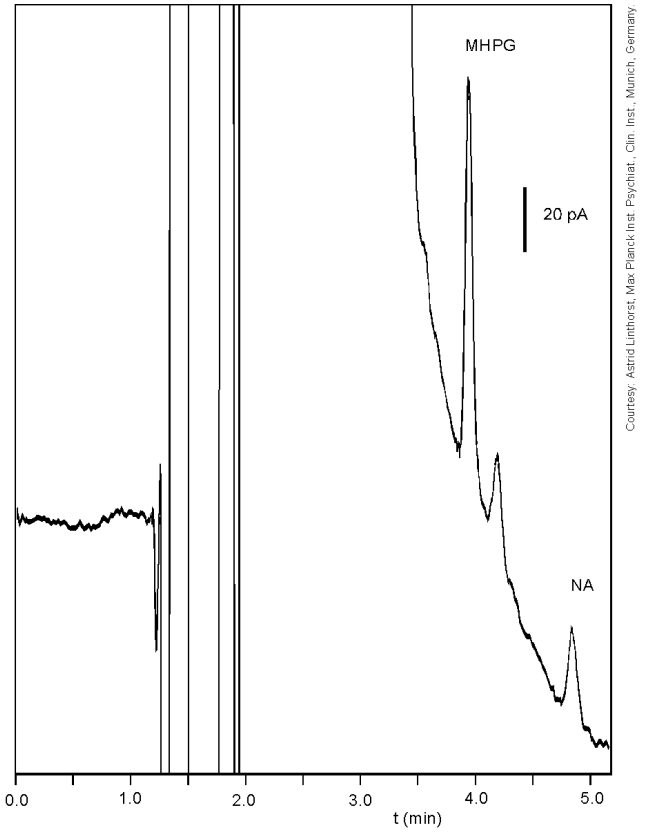
$$c_{LOD} = \frac{3 \times \text{noise pp}}{\text{signal}} \quad c_A = 56 \text{ pmol/L}$$

Minimum detectable amount: 56 pmol/L x 20 µL= 1.12 fmol (10^{-15} mol)

Neurotransmitters in rat tissue



Analysis of rat hyppocampal dialysate 50 µL injection.
Concentrations (amounts) are 185 nM (5.5 pmol) 5-hydroxyindole acetic acid and 154 pM (4.6 fmol) 5-hydroxytryptamine (serotonin).



Analysis of rat preoptic area dialysate 50 µL injection.
Concentrations (amounts) are 10 nM (318 fmol) MHPG and 0.36 nM (11 fmol) Noradrenaline.