# ATTOMOLE DETECTION LIMITS IN MICRO HPLC-ECD DETECTION

THE SMARTEST LC-EC APPLICATIONS FOR NEUROSCIENCE ANALYSIS EVER MASTERMINDED

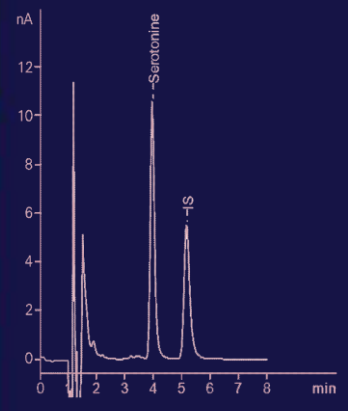
Monoamines and the metabolites
- *Noradrenalin*
- ***Dopamine***
- ***Serotonin***
- ***5-hydroxyindole acetic acid (5-HIAA)***
- ***3,4-dihydroxyphenylacetic acid (DOPAC)***
- *homovanillic acid (HVA)*

OPA derivatized amines and amino acids
- GABA and Glutamate
  - 4-aminobutyrate (GABA)
  - Glutamate (Glu)

Choline and Acetylcholine
- *Choline (Ch)*
- *Acetylcholine (ACh)*

Markers for oxidative stress
- *3-nitro-L-Tyrosine*
- *8-OH-DPAT*

Glutathione and other thiols




## INTRODUCTION

**Characteristic for ECD are the low limits of detection that can be obtained, especially in combination with micro HPLC. Unlike most other detection techniques, detection limits in ECD do not deteriorate in miniaturised systems, they in fact may improve considerably [6]. This not only holds for mass detection limits, which improve (with limited practical use) because of the smaller injection volume, but also for concentration detection limits.**

- Attomol detection limits
- Miniaturization to improve LOD by factor 6 - 10
- Microbore LC-ECD

## Summary

Since the introduction in the early 70'ies, electrochemical detection (ECD) has developed to a reliable, sensitive and selective detection method for HPLC. The technique has found its way in many laboratories in fields of neurochemistry, pharmaceutical chemistry, food chemistry and environmental chemistry. The versatility of the technique is illustrated by many applications [1-5]. In this Technical Note peak heights and signal-to-noise ratios in microbore HPLC are compared to standard HPLC with ECD. Improvements in concentration detection limits by a factor 6-10 are demonstrated. Under the current conditions the minimum detectable amount for dopamine is 160 attomole (25 fg).

*Fig. 1. ALEXYS Analyzer*

## Method

A test mixture of standards consisted of DOPAC, 5-HIAA, HVA (10 nmol/L) and dopamine and 5-HT (0.1 nmol/L). Conclusions are based on multiple injections over several days. The working potential of 650 mV has been selected as the optimum for dopamine. Better detection limits can be obtained at optimum potential for the other compounds, especially in case of HVA.

Table 1

| Conditions | |
|---|---|
| **HPLC** | DECADE ECD |
| **Flow cell** | VT-03 with 2 mm GC WE and Ag/AgCl REF, 25 µm spacer |
| **Ecell** | 650 mV vs. salt bridge Ag/AgCl |
| **Temperature** | 30 °C (separation and detection) |
| **Flow rate** | 1000 or 50 µL/min (using a splitter) |
| **Vinjection** | 20 µL loop, injection programming |
| **LINK** | 0.067 Hz, INT output of D1 |
| **Range** | 1 nA/V |

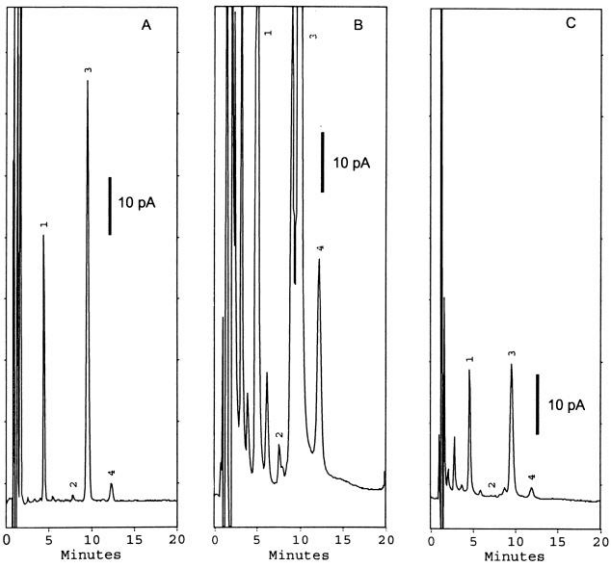



*Fig. 2. Analysis of DOPAC (1), 5-HIAA (3), HVA (4) (10 nmol/L) and dopamine (2) and 5-HT (0.1 nmol/L) using a standard 100 x 4.6 mm column (A), or a 100 x 1 mm column with 20 µL (B) or 1 µL (C) injection volume.*

## Results and discussion

Although the detection limits have been determined for a test mixture detection settings were optimised for dopamine only. The detection limit is defined as the concentration or amount that gives a peak height that is 3 times the peak-to-peak noise.

Concentration detection limits in HPLC-ECD are dependent on a number of parameters such as column efficiency, loadability and injection volume, detector sensitivity, working electrode diameter, and spacer thickness.

To be able to study the chromatographic contribution in detail, the detection parameters must be kept constant. Therefore a comparison of detection limits was made using the same, low dead volume, electrochemical flow cell with different HPLC columns. The noise, which is mainly determined by the size of the working electrode, was found to be the same for standard and micro HPLC (0.15 pA peak to peak noise).

Table 2

| Peak heights (pA) | | | |
|---|---|---|---|
| | std. LC 20µL | µLC 20µL | µLC 1µL |
| DOPAC | 43.2 | 403 | 19.9 |
| Dopamine | 0.97 | 5.8 | 0.24 |
| 5-HIAA | 68.3 | 442 | 21.6 |

Table 3

| Concentration detection limits (pmole/L) | | | |
|---|---|---|---|
| | std. LC 20µL | µLC 20µL | µLC 1µL |
| DOPAC | 104 | 11 | 226 |
| Dopamine | 46 | 8 | 188 |
| 5-HIAA | 66 | 10 | 208 |

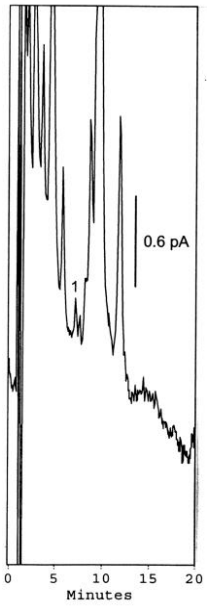



*Fig. 3. Analysis of 0.1 nmol/L (0.1 fmol, 15 fg) dopamine (1) using a 100 x 1 mm column with 1 µL injection volume.*

The optimum detector settings for dopamine were determined by hydrodynamic voltammograms. A series of chromatograms have been obtained at potential settings between 0.4 and 1.0 Volt using 50 mV increments. A working potential of 650 mV gave the best signal-to-noise ratio for dopamine and was therefore used for all further experiments. Consequently, the results for HVA and 5-HT are not considered in detail.

The peak height for 0.1 nmol/L dopamine is 0.97 pA (Fig. 1A, Table I) on a 4.6 mm ID column, which is about 6 times the peak-to-peak noise. The corresponding detection limit is 46 pmole/L (Table II). If the same sample is injected on a 1 mm column a 6 times higher peak is observed, resulting in a factor 6 better detection limit of only 8 pmole/L (Fig. 1B, Table II).

For DOPAC and 5-HIAA the detection limits are somewhat higher in standard LC, probably due to the relatively low working potential (for DOPAC). Especially for DOPAC a considerable improvement of a factor 10 better detection limit is found in micro LC with 20 µL injection volume.

Interestingly, under the working conditions injection of 20 µL on a 1 mm column did not result in overloading. As can be seen from the results of the 1 µL injection (Table I), the peak heights are linear with the injection volume and the column efficiency is the same (Table III). Explanation is found in the 5µm particle size of the column material, which allows higher loadability. Plate numbers are usually somewhat better (about a factor 2) with 3 µm column material.

The result in Table II clearly shows that concentration detection limits in HPLC improve with the sample loadability. This is particularly of interest in micro HPLC. If the loadability is decreased with the square of the column diameter by injecting 1 in stead of 20 µL, a loss in concentration detection limits is found. However, if the higher loadability of micro HPLC is utilised, the detection limits substantially improve. This is unique for electrochemical detection, provided that the flow cell is designed for microbore LC. This holds for the VT03 used in these experiments with an internal volume of only 71 nl.

Table 4

Plate numbers for micro HPLC with ECD

| | µLC 20µL | µLC 1µL |
|---|---|---|
| DOPAC | 3000 | 2700 |
| Dopamine | 4800 | 4700 |
| 5-HIAA | 3800 | 3700 |

Both modifier concentration and pH of the mobile phase are important parameters for tuning of the chromatography in case baseline separation is not achieved for specific microdialysis samples.

## CONCLUSION

**HPLC-ECD has the unique property that it, can be miniaturised with substantial improvement in detection limits. This is illustrated here in comparing a 4.6 and a 1 mm ID column. When injecting 20 µL a concentration improvement of a factor 6 (from 46 to 8 pM) was observed for DA and a factor of 10 for DOPAC. When injecting 1 µL the detection limit for DA was 160 amole (25 fg) (Fig. 2).**

**These data indicate that optimising injection volume, chromatographic and ECD parameters results in impressive detection limits.**

## References

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