Attomole detection limits in micro HPLC-ECD

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Introduction

Characteristic for electrochemical detection (ECD) are the low limits of detection that can be particularly obtained in conjunction with micro HPLC. Unlike most other detection techniques, detection limits in ECD do not deteriorate in miniaturised systems, they in fact may improve considerably [1-6]. Improvements in concentration detection limits by a factor 6-10 compared to standard HPLC-ECD are demonstrated. The minimum detectable amount for dopamine is 160 attomole.

Peak heights and miniaturisation

Peak heights in ECD are described by the limiting current equation. When HPLC is involved, sample dilution over the column should be taken into account as well. Concentration at peak maximum:

$$C_{max} = [\, w\, N^{1/2}] \,/\, [\, t_R\, Fm\, (2\,\pi)^{1/2}\,] \qquad (1)$$

If a micro column is used instead of a standard column, and plate number, retention time and injected amount all remain the same, the concentration of sample eluting from the column is much higher. This effect is predicted by:

$$C_{max\ micro} \,/\, C_{max\ std} = F_{m\ std} \,/\, F_{m\ micro} \qquad (2)$$

Equation (2) shows an increase in peak height of a factor 21 when the flow rate decreases from 1000 to 48 µL/min.
In ECD and using a VT03 wall jet cell, the relation between peak height and concentration is given by the Cotrell equation [6]:

$$i_{lim} = 1.47\ nFC\ [DA/b]^{2/3}\ Fm^{1/3} \qquad (3)$$

Equation (3) shows a decrease in peak height of a factor 2.7 if the flow rate decreases from 1000 to 48 µL/min.

Noise and miniaturisation

In electrochemical detection noise decreases with the working electrode (WE) area until a lower limit where the electronic noise from the controller predominates [6].
If only the column diameter is decreased and the WE area is kept constant, the noise will remain approximately the same in an optimised HPLC-ECD system.

Conclusion

Combining the conclusion of equation 2 and 3 shows that a nett increase in signal of a factor 7.6 can be expected for micro LC, under the given experimental conditions. This is confirmed by our experiments (Fig. 1 A vs. B). At a 10 times smaller injection volume a decrease of a factor 5 can be derived from the same equations. This is demonstrated in Fig. 1A vs. C.
An ALEXYS 100 LC-EC system is used , two pumps are required because of different mobile phase conditions.

Experimental conditions

HPLC	Shimadzu LC10AD pump, SSI pulse dampener, Degasys degasser, Triathlon autosampler
Column	Princeton Spher C18 100Å, 5µm, 100 x 1.0 or 4.6 mm (Princeton, USA)
Flow rate	1 ml/min (standard LC) or 48 µl/min (micro LC)
Mob. phase	50 mmole/L phosphoric acid, 50 mmol/L citric acid, 100 mg/L octanesulphonic acid, 40 mg/L EDTA, 2 mmol/L KCl, brought at pH 4.5 with concentrated NaOH, 8% methanol
Sample	DOPAC, 5-HIAA, HVA (10 nmol/L) and dopamine (0.1 nmol/L)
Injection	20 µL
Detector	DECADE, 1 nA/V, cell current 166 pA and 80 pA (4.6 and 1 mm ID column, resp.)
Noise filter	LINK, 0.067 Hz cut-off frequency
T	30 °C
Cell	VT-03 wall-jet flow cell with 1.9 mm glassy carbon working electrode, spacer thickness 25 µm (Antec Leyden), effective volume 71 nl
E_{cell}	650 mV vs. Ag/AgCl reference electrode (saturated KCl)

Analysis of biogenic amines and acidic metabolites

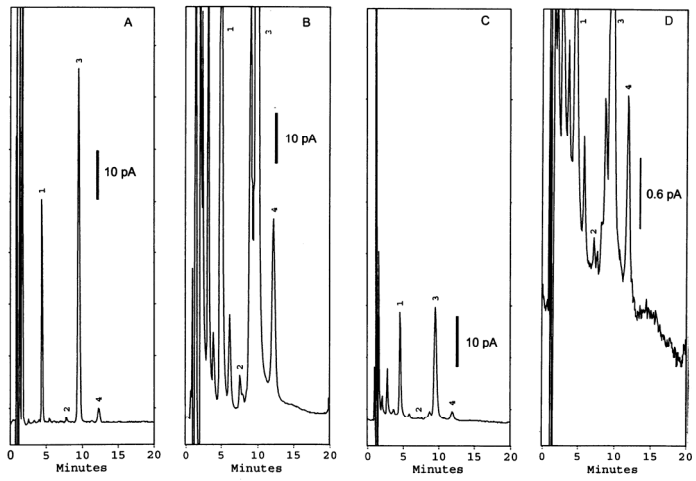

Fig. 1. Analysis of DOPAC (1), 5-HIAA (3), HVA (4) (10 nmol/l) and dopamine (2) (0.1 nmol/l) using a standard 100 x 4.6 mm column (A), or a 100 x 1 mm column with 20 µl (B) or 1 µl (C) injection volume. Close-up of peak 2 (0.1 fmol, 15 fg dopamine) is shown in (D). Noise: 0.15 pA peak to peak.

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