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Using an electrochemical detector with a carbon interdigited-array microelectrode for capillary-column liquid chromatography

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Abstract

In capillary liquid chromatography (LC), an electrochemical detector with a carbon interdigited array (IDA) microelectrode is used effectively. The IDA electrode is found to be more sensitive than the ordinary glassy carbon electrode in capillary LC. Besides the above advantage, using the IDA electrode, a lower background current and noise level were observed. Using a microflow processor at a flow-rate of 4.4 μl min^{-1}, catecholamines and their metabolites (3,4-dihydroxymandelic acid, 3,4-dihydroxyphenylethyleneglycol, vanilmandelic acid, norepinephrine, 3-methoxy-4-hydroxyphenylglycol, 3,4-dihydroxyphenylacetic acid, 3,4-dihydroxybenzylamine, 5-hydroxyindoleacetic acid, vanillacetic acid, homovanillic acid, dopamine and serotonin) were separated in a single run by ion-pair gradient elution. Excellent reproducibility of retention time was obtained with relative standard deviation (R.S.D.) values of 0.5%. The detection limits of catecholamines and their metabolites ranged from 0.19 fmol for 3,4-dihydroxymandelic acid to 3.93 fmol for vanillacetic acid.

Keywords: Detection, LC; Electrochemical detection; Interdigited-array microelectrode; Catecholamines

1. Introduction

Recently, capillary liquid chromatography (LC) has been employed for the determination of biochemical or biomedical compounds because the concentration of target compounds is low, and available sample quantities are small [1–3]. In capillary LC, to obtain optimum performance, it is important to minimize the peak band broadening outside the column, for example, in the detector cell. In microbore high-performance liquid chromatography (HPLC) and micro LC, an electrochemical detector is preferred since high sensitivity is maintained even using a micro-volume cell. Besides the above advantages, the selectivity and sensitivity to some biological amines are much higher than found with UV or fluorescence detectors [4–6].

IDA electrodes have been used as the detector with microbore HPLC and micro LC [7–10]. Repeated oxidization increases the sensitivity of target compounds that have reversible electrochemical reaction properties. In the cell, if the flow velocity of the mobile phase is low, the efficiency of the electrochemical reaction is increased. As a result of these effects, and also because of the minimized dilution effect through the column because of the small column diameter, the sensitivity is increased using a capillary column coupled with the IDA electrode.

Gradient elution in microbore HPLC, micro LC and capillary LC using an electrochemical detector with an IDA electrode has not been reported because of significant baseline drift, fluctuating background

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noise and difficulty in maintaining accurate flow-rates. However, gradient operation is required for separating the biological amines and their metabolites, which have a wide range of chromatographic retention properties.

In this study, the chromatographic characteristics of an IDA electrode and an ordinary glassy carbon electrode were compared using electrochemical detection. We also experimented on gradient elution using an IDA electrode and a capillary column. High sensitivity detection of 12 catecholamine-related compounds has been performed without sacrificing resolution.

2. Experimental

2.1. Reagents

3,4-Dihydroxymandelic acid (DOMA), 3,4-dihydroxyphenylethyleneglycol (DHPG), 3-methoxy-4-hydroxyphenylglycol (MHPG), vanillacetic acid (VLA) and 3,4-dihydroxybenzylamine (DHBA), which was used as an internal standard, were obtained from Sigma (St. Louis, MO, USA). Vanilmandelic acid (VMA), norepinephrine (NE), 3,4-dihydroxyphenylacetic acid (DOPAC), 5-hydroxyindoleacetic acid (5HIAA), dopamine (DA), homovanillic acid (HVA), serotonin (5HT) and sodium 1-octanesulfonate (SOS) were purchased from Tokyo Kasei Kogyo (Tokyo, Japan). The standard samples were dissolved in 0.1 *M* perchloric acid (Kishida Chemicals, Tokyo, Japan). Ethylenediaminetetraacetic acid (EDTA), citric acid, potassium hydroxide (KOH) and phosphoric acid were purchased from Kishida Chemicals (Tokyo, Japan). Methanol (MeOH) was of HPLC grade from Kishida Chemicals. Reagent water was obtained from a Milli-Q water purification system (Waters, Millipore, Milford, MA, USA).

2.2. IDA carbon microelectrode

The IDA carbon microelectrode was purchased from NTT Advanced Technology (Musashino, Tokyo, Japan). It consists of 100 pairs of small electrodes and the channel. The width of the small electrode and of the channel is 5 μm. The width and length of the IDA electrode are each 2 mm.

2.3. Instrumentation

The HPLC system consisted of an autosampler AS640, a binary pump PU610, a thermostated column oven CO630 set at 30°C and an amperometric detector (ED623) with a carbon IDA microelectrode, controlled by a data system VStation (GL Sciences, Tokyo, Japan). The thickness of the gasket for the thin layer flow cell was 25 μm. A microflow processor (Acurate, LC Packings, Amsterdam, Netherlands) connected between the pump and the autosampler was used to split the flow-rate down to the required microflow.

2.4. Chromatographic conditions

In order to study the effect of the flow-rates on the sensitivity of the electrochemical detector, we employed columns of various internal diameters, ranging from 0.3 to 4.6 mm. For each column, the flow-rate of the mobile phase was adjusted to make the linear velocities approximately equal. The columns used for the isocratic elution are shown in Table 1.

The mobile phase for isocratic elution consisted of 50 m*M* citric acid, 50 m*M* phosphoric acid, 100 mg l^{-1} SOS, 50 mg l^{-1} EDTA (pH 3.0) and 5% MeOH. In this isocratic analysis, the potentials of the anodic and cathodic arrays were set at 600 and 0 mV vs. Ag/AgCl, respectively. The injected sample volume contained 835 fmol NE, epinephrine, DHBA, DOPAC and DA, respectively.

Table 1
Characteristics of the HPLC columns used in the experiments

Column diameter (mm)	4.6	1.5	1.0	0.7	0.3
Flow-rate used in experiments	1 ml min^{-1}	100 μl min^{-1}	50 μl min^{-1}	25 μl min^{-1}	4.4 μl min^{-1}
Particle size of packing (μm)	5	5	5	5	3

The packing materials used was Inertsil ODS-3 for all columns of 15 cm length.

Gradient elutions were performed on a 300 μm I.D.×15 cm long column, packed with Inertsil ODS-3, 3 μm diameter packing (GL Sciences). The gradient program in this experiment was as follows. The concentration of solvent B was increased from 0 to 100% in 20 min, then held at 100% until 50 min. Solvent A of the mobile phase consisted of 50 m*M* citric acid, 50 m*M* phosphoric acid, 100 mg l^{-1} SOS and 50 mg l^{-1} EDTA (pH 2.3). Solvent B consisted of 20 (v/v) % MeOH and 80 (v/v) % solvent A. The flow-rate at the start of the gradient was 4.4 μl min^{-1}.

3. Results and discussion

It has been established that the sensitivity of the amperometric detector depends on the flow-rate [11,12]. In this study, we investigated the appropriateness of the IDA electrodes for use in capillary LC. The typical flow-rate effect on the peak area of DHBA is shown in Fig. 1. This is a graph of log (peak area, nC) vs. log (flow-rate, μl min^{-1}) for the range of flow-rates from 4.4 to 1000 μl min^{-1} for the IDA electrode and the ordinary glassy carbon electrode. The dimensions of the ordinary glassy carbon electrode are 2 mm wide and 3 mm long. The response in the ordinary glassy carbon electrode is similar to the results obtained by Prabhu and Anderson [12], that is, the peak area decreases if the flow-rate is decreased. Using the IDA electrode, however, the peak area increases when the flow-rate of the mobile phase is decreased, when using smaller diameter columns. The ordinary glassy carbon electrode is unsuitable for flow-rates below 50 μl min^{-1}. The difference in sensitivity is not due to the difference in cell volume, since we used a gasket of the same thickness, i.e. 25 μm, both for the IDA and the ordinary electrode.

Besides the above effects, the noise level of the IDA electrode was much lower than that of the ordinary glassy carbon electrode. In these measurements, the noise level of the ordinary glassy carbon electrode was 7 pA, whereas the noise level observed

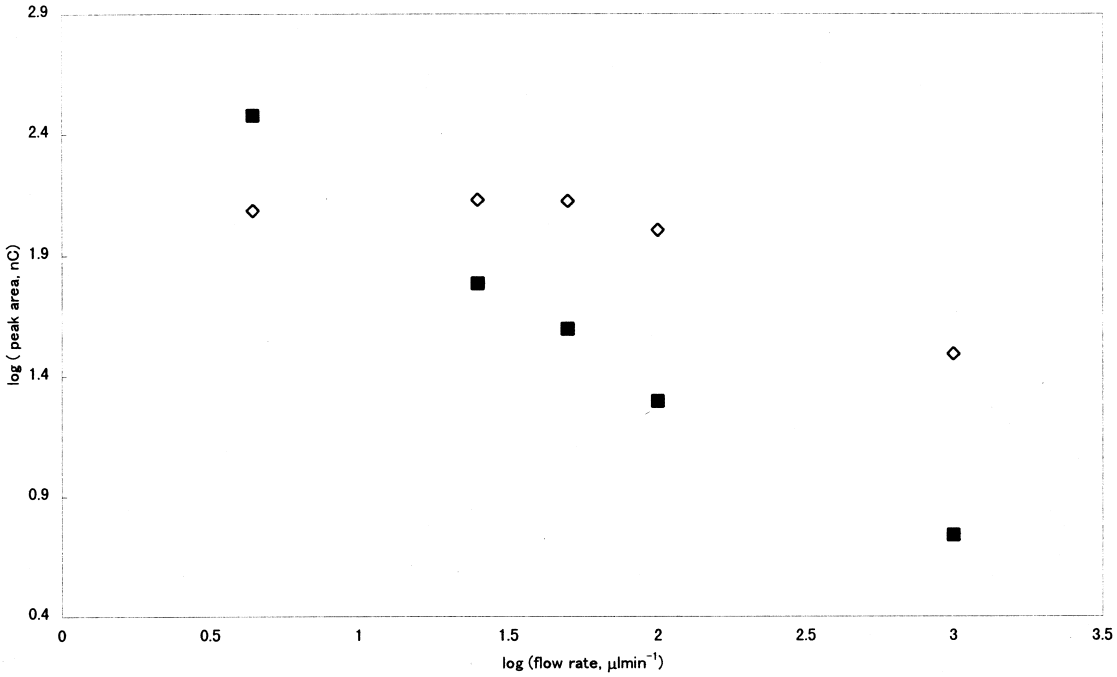

Fig. 1. Peak area (nC) vs. flow-rate (μl min^{-1}), log_{10} coordinates: (■) the IDA electrode; (◇) the ordinary glassy carbon electrode. The noise level of the ordinary glassy carbon electrode was 7 pA, whereas the noise level observed using the IDA electrode was less than 1 pA (for other conditions, see Section 2).

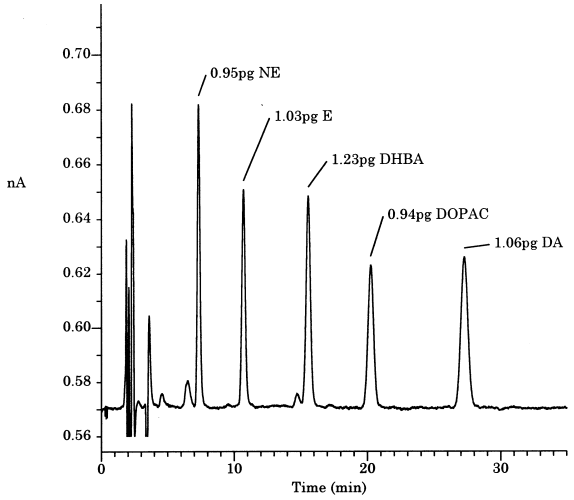

Fig. 2. Chromatogram of NE, E, DHBA, DOPAC and DA, with isocratic elution. A 150×0.3 mm I.D. capillary column was used at a flow-rate of 4.4 μl min^{-1}. The injected sample volume was 0.1 μl. The anodic and cathodic array electrodes in the IDA were potentiostated at 600 and 0 mV vs. Ag/AgCl, respectively (for other conditions, see Section 2).

using the IDA electrode was less than 1 pA. The IDA electrodes in capillary LC are expected to achieve low detection limits for catecholamines and their metabolites because of the high current and low noise level of the IDA electrode. A chromatogram of the isocratic elution of NE, epinephrine, DHBA, DOPAC and DA is shown in Fig. 2. The flow-rate was 4.4 μl min^{-1} and the volume of sample injected was 0.1 μl, containing 947, 1026, 1233, 941, and 1062 fg of each compound, respectively. The potentials of the anodic and cathodic arrays were set at 600 and 0 mV vs. Ag/AgCl. In this measurement, the noise level was about 1 pA. Peak heights of 112 pA for NE, 80 pA for epinephrine, 78 pA for DHBA, 53 pA for DOPAC and 56 pA for DA were obtained. The detection limits of NE, epinephrine, DHBA, DOPAC and DA, calculated from the peak heights and noise level, were 25.4, 38.5, 47.4, 53.3 and 56.9 fg, respectively. Here, the S/N ratio was assumed to be three. In the isocratic experiment, five compounds

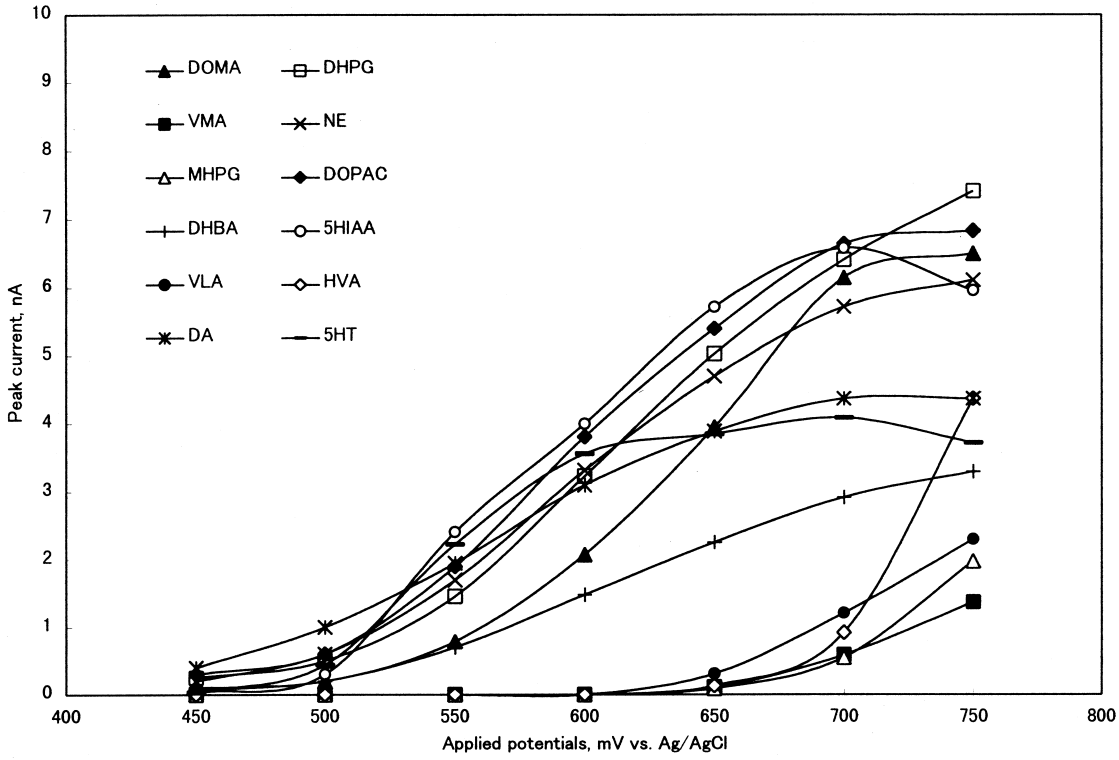

Fig. 3. Hydrodynamic voltammograms for several catecholamines and their metabolites using the IDA electrode with gradient elution. The potential of the cathodic array was set at 0 mV vs. Ag/AgCl. and 3 ng of each sample were injected (for other conditions, see Section 2).

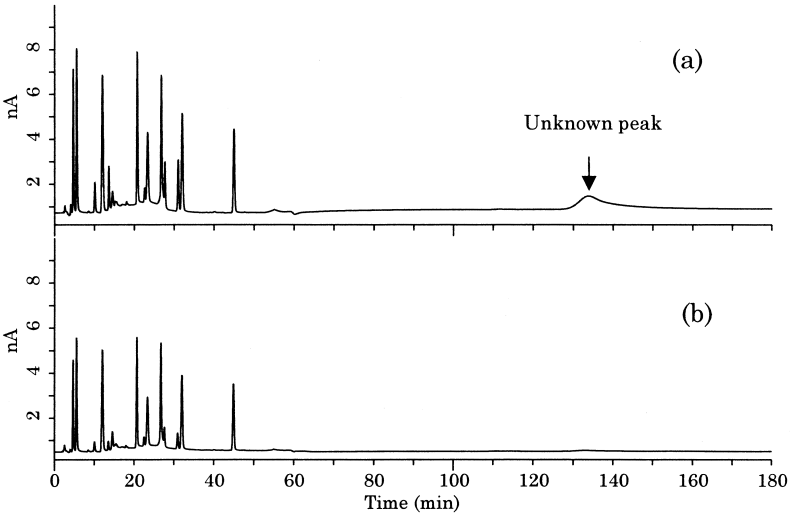

Fig. 4. Comparison of the chromatograms obtained at (a) 750 mV and (b) 700 mV of the anodic array potential. The gradient elution was completed 50 min after the starting time. Then, the mobile phase composition was returned to the initial conditions, which took 3 min. The baseline was then observed under the initial solvent conditions until 180 min (for other conditions, see Section 2).

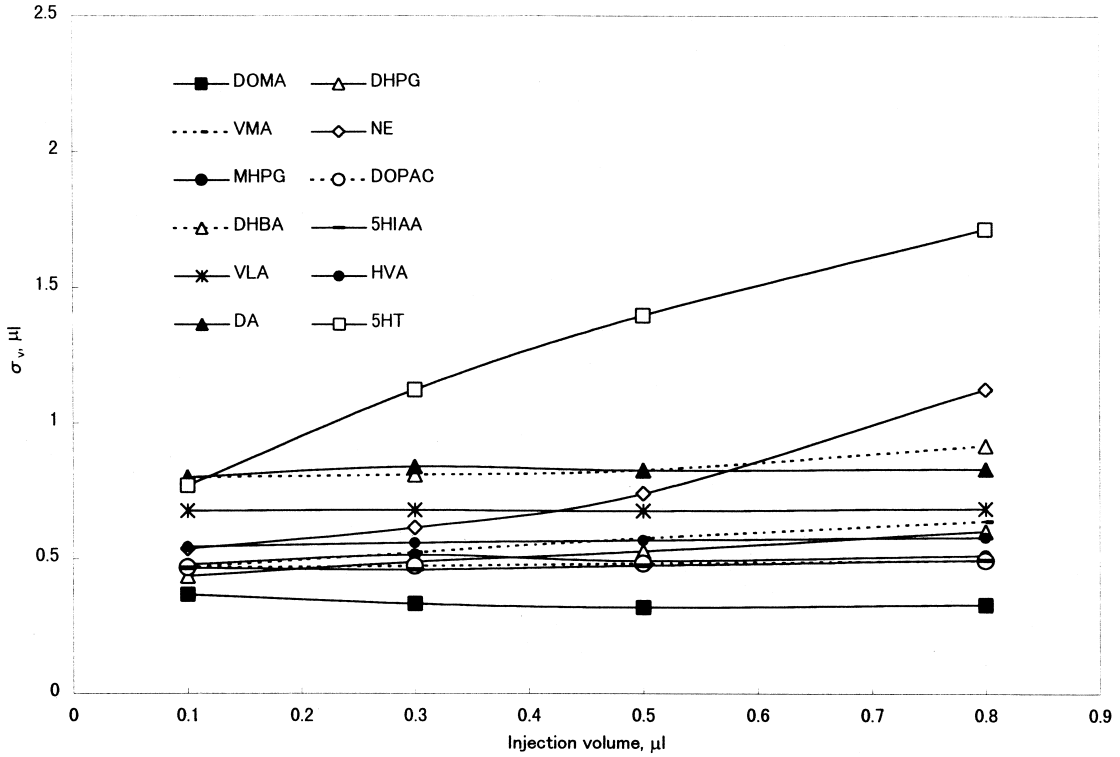

Fig. 5. Effect of increasing the injection volume on the σ_v of catecholamines and their metabolites. The capillary column (150×0.3 mm I.D.) was used at a flow-rate of 4.4 μl min^{-1}, in gradient elution mode. The concentration of each compound was 200 ng ml^{-1}, dissolved in 0.1 *M* perchloric acid (for other conditions, see Section 2).

were detected with high sensitivity and good resolution in a single run, as illustrated in Fig. 2.

In general, isocratic elution has been employed in association with HPLC with electrochemical detection (LCEC) for measuring biological amines and their metabolites. A gradient elution system is possible in conventional LCEC, whereas gradient elution in capillary LCEC may cause significant baseline drift at high detection gain, fluctuating background noise and difficulty in maintaining accurate flow-rates. In this study, ion-pair gradient elution in capillary LCEC was tried using a system comprising a microinjection autosampler, a microflow processor with a built-in mixer [3,13] and an amperometric detector with the above-mentioned high-performance IDA electrode.

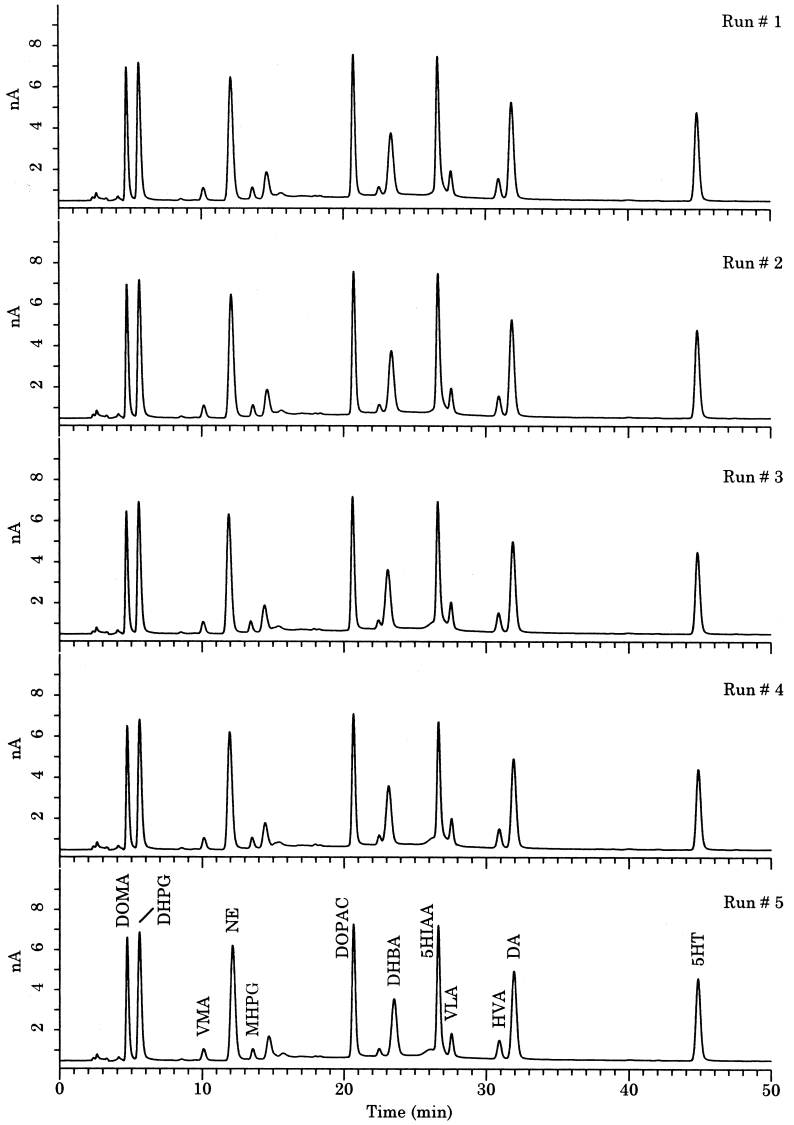

Fig. 6. Study of the reproducibility of retention times using ion−pair gradient elution. The flow-rate was 4.4 μl min^{-1} and the volume of sample injected was 0.3 μl, containing 60 pg of each compound. The potentials of the anodic and cathodic arrays were set at 700 and 0 mV vs. Ag/AgCl, respectively (for other conditions, see Section 2).

Hydrodynamic voltammograms (HDV) for several catecholamines and their metabolites, obtained using the IDA electrode and a gradient elution mobile phase, are shown in Fig. 3. The potential of the cathodic array was set at 0 mV vs. Ag/AgCl and the potential of the anodic array was varied over the range from 450 to 750 mV vs. Ag/AgCl. The optimized potential of the anodic array for the gradient elution was 700 mV vs. Ag/AgCl because potentials higher than 700 mV vs. Ag/AgCl caused remarkable baseline drift and the detection of unknown peaks. These phenomena are shown in Fig. 4.

To circumvent the need for sample splitting during injection and to avoid the loss of valuable sample, increased injection volume due to on-column focusing (V_{max}) was investigated [14–17]. The value of V_{max} was determined using volume peak standard deviation (σ_v) data. σ_v was calculated as follows: σ_v=peak width at half height in volume units/2.354. This theory was proposed by Mills et al. [18]. In Fig. 5, the effect of increasing injection volumes of catecholamines and their metabolites on σ_v are shown. Peak dispersion due to increasing injection volume was significantly noted for NE and 5HT. The cause of the NE and 5HT peak band broadening is thought to be the fact that the mobile phase was diluted with the sample solvent, especially when larger volumes of sample were injected. These two types of compounds were particularly influenced by this effect. Ultimately, a 0.3-μl sample was injected directly onto the packed 300 μm I.D. capillary column, using on-column focusing. This allows for effective sample focusing without any measurable peak dispersion and the direct use of a microinjection autosampler.

In Fig. 6, the reproducibility of five consecutive injections using an autosampler is shown. DOMA, DHPG, VMA, NE, MHPG, DOPAC, DHBA, 5HIAA, VLA, HVA, DA and 5HT were separated by the gradient elution. In this gradient elution, the potentials of the anodic and cathodic arrays were set at 700 and 0 mV vs. Ag/AgCl. The volume of sample injected was 0.3 μl, containing 60 pg of DOMA, DHPG, VMA, NE, MHPG, DOPAC, DHBA, 5HIAA, VLA, HVA, DA and 5HT, respectively. The sequence of the analysis is shown in Fig. 6 as the run number. The retention times of some compounds apparently varied, however, as the result of the calculation, the R.S.D. values of the retention times of all peaks were within 0.5% (n=5). The detection limits of DOMA, DHPG, VMA, NE, MHPG, DOPAC, DHBA, 5HIAA, VLA, HVA, DA and 5HT, calculated from the peak heights and the noise level, are shown in Table 2.

Table 2
Limits of detection of catecholamines and their metabolites during ion-pair gradient elution

Compound	LOD[A]		Compound	LOD[A]	
	(fg)	(fmol)		(fg)	(fmol)
DOMA	35.5	0.19	DHBA	80.5	0.37
DHPG	33.9	0.20	5HIAA	39.7	0.21
VMA	397	2.00	VIA	921	3.93
NE	38.0	0.22	HVA	220	1.21
MHPG	844	3.71	DA	51.8	0.27
DOPAC	35.1	0.21	5HT	54.0	0.25

[a] S/N=3.

In the gradient experiment, twelve catecholamine-related compounds were separated and detected with high sensitivity (e.g. 0.19 fmol for DOMA to 3.93 fmol for VLA), using a 0.3-mm diameter capillary column connected to the electrochemical detector mounting the IDA electrode. The reproducibility of the retention time was within 0.5%, as R.S.D.

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