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Journal of Chromatography A, 722 (1996) 151–156

JOURNAL OF CHROMATOGRAPHY A

Analysis of overlapped chromatographic peaks by multi-channel electrochemical detection

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Abstract

Quantitative deconvolution of unresolved chromatographic peaks by multi-channel electrochemical detection was successfully performed. An electrochemical detector with four cells was employed. A regenerated current–voltage spectrum of three analyte components was obtained and used for peak deconvolution of the unresolved chromatographic peak. The overlapped peaks of three components, 5-hydroxyindole-3-acetic acid (5HIAA), deoxyepinephrine (DEP) and 3,4-dihydroxyhydrocinnamic acids (DHCA), was deconvoluted into the respective peaks of the components. The recoveries were 100.2% (5HIAA), 99.2% (DEP) and 99.7% (DHCA). This method could be used for the analysis of overlapped peaks in high-performance liquid chromatography with electrochemical detection.

1. Introduction

The determination of multiple components eluted together has been a long-term problem in chromatography and addressed by many workers [1–5]. There are various difficulties in achieving such chromatographic separations, such that multi-component samples cannot be separated into simple components completely, even with carefully optimized chromatographic conditions.

Dual-wavelength ultraviolet detection is widely used to generate absorption ratios as a relatively non-specific method for the characterization of peak purity. Poorly resolved chromatographic peaks have been mathematically resolved to give all components peaks using a multi-channel multiwavelength spectrophotometric UV detector and a personal computer [3]. Quantitative deconvolution of chromatogram peaks with extremely low UV absorption has been demonstrated in the analysis of an anaesthetic and its metabolite in serum [5]. Using a variety of models in UV detection, several methods for deconvoluting overlapped peaks have been discussed [6–9].

Using electrochemical detection with high-performance liquid chromatography (HPLC), several investigators have been trying to determine the levels of neurotransmitters and their metabolites in cerebrospinal fluid, blood and urine [10–15]. However, it seems extremely difficult to separate them completely in a single chromatographic separation.

This paper describes a method for the quantitative deconvolution of overlapped peaks using a multi-channel electrochemical detector with HPLC. This method needs the characteristic current–voltage spectrum of respective analyte component. We use the regenerated current–voltage in four analytical cells which were con-

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0021-9673/96/$15.00
SSDI 0021-9673(95)00830-6

nected in series. The regenerated current–voltage is the oxidative or reductive current that is observed when selected potentials are applied to each analytical cell of the detector.

2. Theory

A chromatogram obtained from a multi-channel electrochemical detector is defined as a function of applied potential, V, and retention time, t, as $C_s(V, t)$ in three-dimensional chromatographic domains. For a multi-component sample, the chromatogram is expressed as a summation over each individual component, i, as follows:

$$C_s(V, t) = \sum C_i(V, t) + e \quad (1)$$

where e represents the error due to noise. The chromatogram of each individual component, i, is expressed as

$$C_i = c_i f_i(V, t) \quad (2)$$

where $f_i(V, t)$ represents the chromatogram of a unit amount of the component i obtained by injecting a standard sample of the component i and c_i is a coefficient of quantity for the component i.

Then, the chromatogram of a multi-component sample at the applied potential V_j is expressed as $C_s(V_j, t)$, and the regenerated current spectrum that is observed as a function of applied potentials at time t_k is expressed as $C_s(V, t_k)$. Thus, the observed current spectrum of a multi-component sample at time t_k can be expressed by using Eqs. 1 and 2 as follows:

$$\begin{vmatrix} C_s(V_1, t_k) \\ C_s(V_2, t_k) \\ \vdots \\ C_s(V_j, t_k) \end{vmatrix} = \begin{vmatrix} f_1(V_1, t_k) & f_2(V_1, t_k) & \cdots & f_n(V_1, t_k) \\ f_1(V_2, t_k) & f_2(V_2, t_k) & \cdots & f_n(V_2, t_k) \\ \vdots & \vdots & & \vdots \\ f_1(V_j, t_k) & (f_2(V_j, t_k) & \cdots & f_n(V_j, t_k) \end{vmatrix} \begin{vmatrix} c_1 \\ c_2 \\ \vdots \\ c_n \end{vmatrix} + e \quad (3)$$

where $C_s(V_1, t_k), C_s(V_2, t_k), \ldots, C_s(V_j, t_k)$ represent observed regenerated currents for all cells which carry the potentials $V_1, V_2, \ldots, V_j$, and e is the error due to noise. The isolated cell current for the components $i, \ldots, n$, i.e., $c_1, c_2, \ldots, c_n$, could be determined by algebraically solving Eq. 3 by applying the multiple component analysis technique to the regenerated current–voltages obtained at time t_k. Eq. 3 consists of serial of independent equations for the time t_k and represents the cross-section along the potentiated cell and current axes intersecting the time axis at t_k, as shown in Fig. 1A–C.

In principle, the compositional ratio of as many j components might be determined as their individual current spectra that could be obtained. To correct for the error inherent in data collection, it is preferable to employ the least-squares method for determining $C_1, \ldots, C_n$. Once isolated currents for the respective components at time t_k have been obtained, isolated chromatograms at any cell for the components can be numerically derived from Eq. 2 by inserting the V_j of interest. Then peak integration can be performed independently as with a single peak chromatogram.

The pattern of a characteristic regenerated current–voltage spectrum could be achieved if appropriate different voltages were applied to the respective cells. It should be noted that there are some time lags in peak retention, because chromatograms are monitored at different cells connected in series.

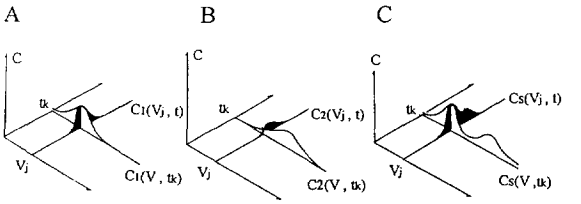

Fig. 1. Schematic representation of a three-dimensional chromatogram of (C) a multi-component sample and (A and B) its components.

3. Experimental

3.1. Reagents

The tested materials, 5-hydroxyindole-3-acetic acid (5HIAA), deoxyepinephrine (DEP) and 3.4-dihydroxyhydrocinnamic acid (DHCA), and 1-heptanesulfonic acid, citric acid monohydrate, sodium dihydrogenphosphate dihydrate, phosphoric acid and Na_2EDTA were obtained from Wako (Osaka, Japan) and methanol and acetonitrile (HPLC grade) from Kanto Chemical (Tokyo, Japan). Water was purified from tap water using a Milli-Q/R and Mill-Q water purification system (Millipore, Bedford, MA, USA).

3.2. Instrumentation

The chromatographic system consisted of a Model 880 intelligent pump (JASCO, Tokyo, Japan), an L-ODS analytical column (150 × 4.6 mm I.D.) (Chemicals Inspection and Testing Institute, Tokyo, Japan), an electrochemical detector including a guard cell (Model 5021) and four analytical cells (Model 5011) (ESA, Bedford, MA, USA). Four cells were employed in the order cell 1 to cell 4. An SC77 signal cleaner (System Instruments, LDH, Tokyo, Japan) was employed to improve the S/N of the signals from the electrochemical detector. Two LC-100 data analytical systems (Yokogawa, Tokyo, Japan) and a PC-9801-RX personal computer (NEC, Tokyo, Japan) were used for data analysis. The chromatographic data was saved at once and subsequently analysed. The time constant of data points was 0.2 s. A scheme of the peak deconvolution system which was used in this experiment is shown in Fig. 2.

3.3. Experiments

The column temperature was maintained at 25°C with a water-jacket. The flow-rate was 1.0 ml/min. The mobile phase used was 0.03 M citric acid monohydrate–0.04 mM phosphate–5.15 mM 1-heptanesulfonic acid–0.11 mM Na_2EDTA–7.5% acetonitrile–4% methanol (pH 3.10). The standard materials were prepared at concentrations of 0.992 mg/l (5HIAA), 1.564 mg/l (DEP) and 1.125 mg/l (DHCA) in 0.2 M perchloric acid. A 5.0-μl sample volume was injected into the column. To minimize the solvent noise, a potential of 400 mV was applied to the guard cell.

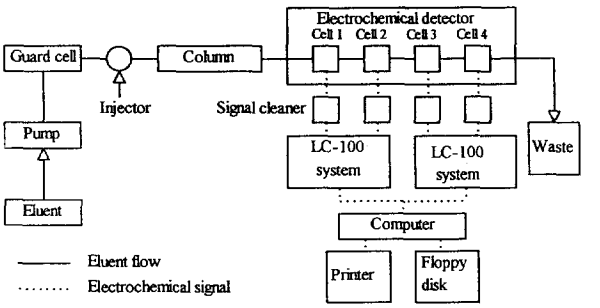

Fig. 2. Schematic diagram of multi-channel electrochemical detection for peak deconvolution.

Conventional current–voltage curves of 5HIAA, DEP and DHCA were monitored by injecting each component into the column and monitoring at the first cell in the potential range from −500 to 500 mV. To obtain the appropriate regenerated current–voltage spectrum for the peak deconvolution, each analyte component was injected into the column, then the oxidative or reductive current was monitored at the respective cell. The current was detected at the peak top and was expressed as μA/mol.

3.4. Calculation and deconvolution

For deconvoluting unresolved chromatographic peaks of 5HIAA, DEP and DHCA, in Eq. 3, i was 3, j was 4, $C_s(V_1, t_k)$, $C_s(V_2, t_k)$, $C_s(V_3, t_k)$ and $C_s(V_4, t_k)$ were the current values for three individual chromatograms at t_k, and f_1, f_2 and f_3 were the values of current per mole. The lag in elution time of the related peaks was corrected. The coefficients of quantity of 5HIAA, DEP, DHCA, c_1, c_2 and c_3, respectively, were calculated point by point over all elution time points using Eq. 3 with a least-squares method. Then peak integration was performed independently on 5HIAA, DEP and DHCA.

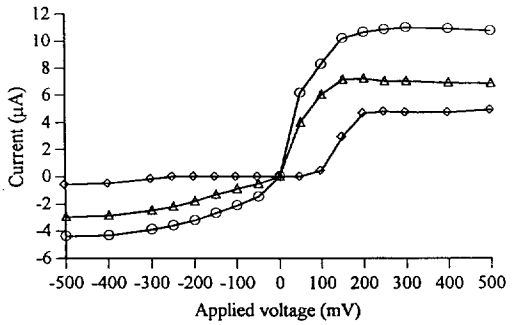

Fig. 3. Conventional current–voltage curves for (◇) 5HIAA, (○) DEP and (△) DHCA.

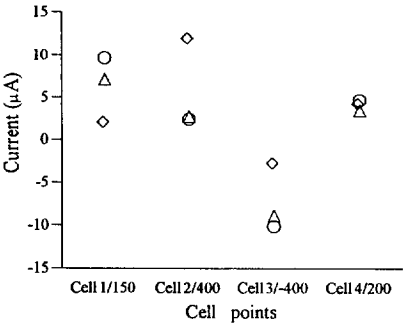

Fig. 4. Regenerated current–voltage spectra of (◇) 5HIAA, (○) DEP and (△) DHCA. The mobile phase was 0.03 *M* citric acid monohydrate–0.04 m*M* phosphate–5.15 m*M* 1-heptanesulfonic acid sodium salt–0.11 m*M* Na_2EDTA–7.5% acetonitrile–4% methanol in pH 3.10. The voltages applied to the first, second, third and fourth cells were 150, 400, −400 and 200 mV, respectively.

4. Results

Fig. 3 shows the conventional current–voltage curves for 5HIAA, DEP and DHCA. The curves were applicable for the choice of voltage to detect multiple components, selecting the different respondent currents of the components. It was expected that multiple components might be detected at the different cells. However, the overlapped peaks could not always be detected as a single peak from the ratio.

Under the chromatographic conditions the analyte components showed different electrochemical behaviour, i.e., the oxidative or reductive potential of the component was different to each other. Referring to the conventional current–voltage curve, suitable series of applied voltages were selected. An appropriate current spectrum for the deconvolution of overlapped peaks of 5HIAA, DEP and DHCA was found when a series of voltages, 150, 400, −400 and 200 mV, were applied to the first, second, third and fourth cells. Fig. 4 shows the spectra of regenerated current–voltage obtained. These spectra showed characteristic enough for the deconvolution of the overlapped peaks.

Fig. 5 shows the unresolved chromatographic peaks of 5HIAA, DEP and DHCA monitored in the four cells. For convenience, the chromatogram of the third cell [Fig. 5(3)] has been inverted because the signal was reductive. As shown in the chromatograms, there are differences in the peak shapes. This is important because the process in deconvolution depends on different responses between the analyte components.

The elution time of the related peak was different between the cells; the time lags were 0.4, 1.6 and 0.4 s between the first and second cells, the second and third cells, and the third and fourth cells, respectively. Before the calculation, we corrected the elution time lags to agree with the retention time of the first cell. This correction was necessary for accurate quantification of peak areas. The overlapped peaks were

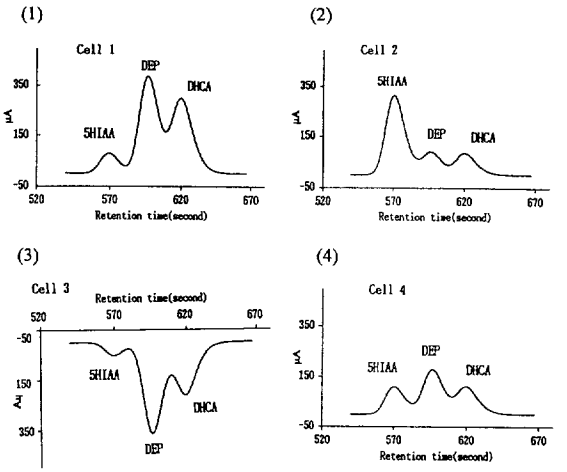

Fig. 5. Overlapped chromatographic peaks of 5HIAA, DEP, DHCA observed in the four cells. The chromatogram of the third cell is inverted.

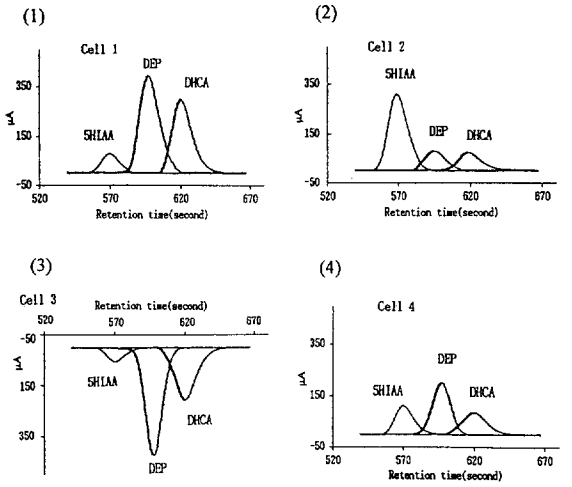

Fig. 6. Deconvoluted chromatograms of Fig. 5.

successfully deconvoluted into the individual component peaks (Fig. 6). The recoveries of the deconvoluted peaks were calculated with respect to the amounts injected.

Table 1 summarizes the resulted yields obtained by this method in comparison with conventional methods. The recoveries obtained by the proposed method are 100.2, 99.2 and 99.7% (R.S.D. = 0.7, 0.3 and 0.8%, respectively) for 5HIAA, DEP and DHCA, respectively, whereas the minimum perpendicular method gave 91.29, 101.2 and 106.5% (R.S.D. = 4.9, 7.8 and 5.5%) and the tangential skimming-dropping method gave 62.3, 48.0 and 48.2% (R.S.D. = 18.5, 24.8 and 12.2%), respectively.

5. Conclusion

The regenerated current–voltage spectra of the components were characteristic enough to deconvolute the overlapped peaks into individual peaks. Using a model of overlapped chromatograms, peak deconvolution was successfully performed in multi-channel electrochemical detec-

Table 1
Comparison of quantitative accuracy with different methods

Method	Cell	5HIAA		DEP		DHCA	
		Observed (fmol)	Recovery (%)	Observed (fmol)	Recovery (%)	Observed (fmol)	Recovery (%)
Standards		25.9		38.4		30.9	
Multi component analysis	Cell 1	26.1	100.8	38.0	99.1	30.5	98.9
	Cell 2	26.0	100.4	38.0	99.1	30.6	99.2
	Cell 3	25.6	98.9	38.3	99.8	30.8	99.7
	Cell 4	26.1	100.6	37.9	99.8	31.1	100.9
	Mean	25.9	100.2	38.1	99.2	30.8	99.7
	R.S.D. (%)	0.7	0.7	0.3	0.3	0.8	0.8
Perpendicular dropping	Cell 1	21.8	84.1	36.3	93.7	29.7	96.4
	Cell 2	24.7	93.1	42.9	111.8	33.8	109.7
	Cell 3	24.5	94.7	35.6	92.8	33.5	108.6
	Cell 4	24.1	92.9	40.9	106.5	34.3	111.2
	Mean	23.8	91.2	38.9	101.2	32.8	106.5
	R.S.D. (%)	4.9	4.9	7.8	7.8	5.5	5.5
Tangent skimming	Cell 1	12.6	48.5	22.4	58.5	12.7	41.1
	Cell 2	20.7	79.8	10.7	27.8	17.5	56.8
	Cell 3	16.6	64.4	20.4	53.3	15.4	50.0
	Cell 4	14.6	56.5	20.1	52.4	13.8	44.9
	Mean	16.1	62.3	18.4	48.0	14.9	48.2
	R.S.D. (%)	18.5	18.5	24.8	24.8	12.2	12.2

The yields of the deconvoluted peaks are compared with those of conventional methods for typical overlapped peaks of 5HIAA, DEP and DHCA.

tion and also in multi-channel spectrophotometry. In multi-channel electrochemical detection, it is easy to select a characteristic regenerated current–voltage spectrum. The combined technique of multi-channel electrochemical detection and multiple component analysis will be useful for the determination of components hidden in overlapped peaks.

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